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<b>(21) International Application Number:</b> PCT/GB91/00510 <b>(22) International Filing Date:</b> 2 April 1991 (02.04.91)  <b>(30) Priority data:</b> 9007052.5                      29 March 1990 (29.03.90)                      GB  <b>(71) Applicant (for all designated States except US):</b> SKUA INVESTMENTS LIMITED [GB/GB]; 19/21 Circular Road, Douglas, Isle of Man (GB).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only) :</b> CHO, Young, W. [US/AU]; Unit 20, 1 High Street, Freemantle, W.A. 6160 (AU). FLYNN, Michael, John [GB/GB]; Hunterscombe, Dorking Road, Leatherhead, Surrey KT22 8JT (GB). SHEPHERD, Thomas, Smith [GB/GB]; 20 Turpine Rise, Windlesham, Surrey GU20 6NG (GB).		<b>(74) Agents:</b> SHEARD, Andrew, Gregory et al.; Kilburn & Strode, 30 John Street, London WC1N 2DD (GB).  <b>(81) Designated States:</b> AT (European patent), AU, BE (European patent), BG, CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), GR (European patent), HU, IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, PL, RO, SE (European patent), SU, US.  <b>Published</b> <i>With international search report.          Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> PHARMACEUTICAL FORMULATIONS		
<b>(57) Abstract</b>  A pharmaceutical formulation comprises a biologically active material such as insulin, erythropoietin, calcitonin and growth hormone, and, associated with it, a phospholipid for forming a material which participates in the alpha-glycerol or other pathway for the formation of lecithins which are found in the intestinal epithelial cell. Biologically active proteins orally administered in such a formulation are bioavailable and bioactive.		

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## PHARMACEUTICAL FORMULATIONS

This invention relates to pharmaceutical formulations. More particularly, the invention relates to orally or rectally administrable formulations of biological active material, particularly proteinaceous materials.

Medical practice has for many years prescribed or advised the administration of many biologically active materials for the treatment or prophylaxis of a wide variety of diseases or conditions. One of the most well known, but by no means the only, prescribed biologically active proteinaceous material is insulin, which is used for the control of diabetes.

Possibly the easiest method of taking any medication is oral ingestion. Such route of administration, which may be by means of syrup, elixir, tablets, capsules, granules, powders or any other convenient formulation, is generally simple and straightforward and is frequently the least inconvenient or unpleasant route of administration from the patient's point of view. It is therefore unfortunate, from the point of view of medical treatment and prophylaxis, that the preferred route of administration of proteinaceous medicaments and other biologically active materials involves passing the material through the stomach, which is a hostile environment for many materials, including proteins. As the acidic, hydrolytic and proteolytic environment of the stomach has evolved efficiently to digest proteinaceous materials into amino acids and oligopeptides for subsequent anabolism, it is hardly surprising that very little or any of a wide variety of biologically active proteinaceous material, if simply taken orally, would survive its passage through the stomach to be taken up by the body in the small intestine.

The result, as many diabetics can testify, is that many proteinaceous medicaments have to be taken parenterally, often by subcutaneous, intramuscular or intravenous injection, with all the inconvenience, discomfort and difficulties of patient compliance that that entails.

1 This is not an isolated problem, as diseases needing control by the  
2 administration of proteinaceous material can be very widespread. Diabetes  
3 mellitus, for example, claims a large number of sufferers in many countries of  
4 the world. Partly because of the large number of patients suffering from  
5 diabetes of one form or another, there is a need to develop oral formulations of  
6 insulin which are somehow protected against the hostile environment of the  
7 stomach. Although various prior attempts at developing such formulations have  
8 been made, the applicants are not aware of any prior composition that has to  
9 date been commercialised to any appreciable degree. Prior proposals of which  
10 the applicants are aware are as follows.

11

12 WO-A-8701035 relates to parenterally administrable formulations of fat-soluble  
13 drugs and vitamins; the formulations comprise 'pseudomicelles'.

14

15 WO-A-8705505 discloses orally ingestible compositions of insulin coated onto  
16 solid particles from an aqueous preparation; the insulin-coated particles are  
17 themselves then coated with lipid.

18

19 US-A-4849405 discloses orally ingestible compositions of insulin; the  
20 compositions are described as being two-phase preparations, and it appears that  
21 both phases are aqueous, with the phases effectively being kept separate by a  
22 coacervate system.

23

24 EP-A-0140085 discloses drug-containing lipid vesicle preparations.

25

26 Shichiri *et al* (*Acta diabet. lat.* 15 175-183 (1978)) disclose  
27 water-in-oil-in-water insulin micelles.

28

29 US-A-4784845 and US-A-4816247 disclose emulsion compositions for the  
30 parenteral administration of hydrophobic drugs.

31

32 JP-A-55017328 discloses water-in-oil-in-water emulsions containing insulin, for  
33 oral ingestion.

1 EP-A-0366277, published on 2nd May 1990, relates to improved  
2 pharmaceutical formulations that can be delivered orally or rectally. More  
3 specifically, EP-A-0366277 teaches a pharmaceutical formulation comprising a  
4 microemulsion having a hydrophilic phase and a hydrophobic phase, wherein  
5 (A) the hydrophilic phase is dispersed in the hydrophobic phase, (B) the  
6 hydrophilic phase comprises a biologically active material and (C) the  
7 hydrophobic phase contains chylomicra or material from which chylomicra are  
8 formed *in vivo*. The hydrophilic phase can contain a physiologically compatible  
9 solvent for the biologically active material, such as water. It is suggested that  
10 the biologically active substance, when administered in association with  
11 chylomicra or the constituents of chylomicra, is targeted to the villae and  
12 microvillae of the intestinal wall, from where it is secreted into the lacteals and  
13 intestinal lymph and then drained into the thoracic duct and, ultimately, the  
14 circulating bloodstream.

15  
16 As is known, chylomicra comprise a lipid/cholesterol core or matrix, surrounded  
17 by a membrane comprising a phospholipid monolayer which is studded with  
18 proteins (Redgrave in *Gastrointestinal Physiology IV*, International Review of  
19 Physiology, Volume 28, 103-130, Young, J. A., Ed., University Park Press,  
20 Baltimore, 1983). It can thus be seen that the prior European patent application  
21 provides the biologically active material in the hydrophobic core.

22  
23 This invention relates to a different approach to solving the problem of orally  
24 (or rectally) administering biologically active compounds, particularly proteins.  
25 It has been discovered that proteinaceous compounds, including but not being  
26 limited to insulin (whether bovine, porcine, human or other), growth hormone  
27 (whether human or other), calcitonin (whether salmon or other), erythropoietin  
28 (whether human or other) can be orally delivered in association with one or  
29 more phospholipids or other compounds involved in the formation of lecithin  
30 ("a lecithin precursor"). The association may be referred to as a  
31 "phospholipo-protein" (such as "phospholipo- insulin"), when the biologically  
32 active compound is proteinaceous.

33

1 According to a first aspect of the invention, there is provided a pharmaceutical  
2 composition comprising a proteinaceous or other biologically active compound  
3 and a lecithin or a lecithin precursor.

4  
5 The proteinaceous compound may be replaced by (or supplemented with) any  
6 other biologically active compound. The mode of action in such cases is  
7 believed to be analogous to that set out above. The biologically active  
8 compound and the lecithin precursor will generally be in some form of  
9 association with each other.

10  
11 Lecithin can integrate into chylomicra, particularly into their membranes. The  
12 use of other compounds, or precursors of them, which can similarly so integrate  
13 is also within the scope of the invention. The discussion in this specification  
14 relating to lecithin or its precursors may be taken to apply to this more general  
15 class of compounds *mutatis mutandis*.

16  
17 The lecithin precursor may form lecithin within the intestinal epithelium of man  
18 or other animals, and so the proteinaceous compound will be in association with  
19 the lecithin as formed as a lecithin-protein complex (such as lecithin-insulin).  
20 Lecithin formed in this way within the intestinal epithelium may form the  
21 surface membrane of chylomicra as well as covering up to 80% of the surfaces  
22 of apolipoproteins, such as apoprotein-A, -B, -C and -E. Thus, optionally using  
23 appropriate absorption enhancers for the phospholipo-protein complex, the  
24 complex may be absorbed into the intestinal epithelium; lecithin is then  
25 synthesised (and the complex then becomes a lecithin-protein complex); and the  
26 lecithin may then cover chylomicron cores as well as those apoproteins attached  
27 to chylomicra. The lecithin may then be released into lymphatic vessels, drained  
28 into the thoracic duct (and those lecithin-protein complexes still attached to  
29 chylomicra may form part of the remnant chylomicra), channelled into the liver  
30 and from there released into the circulating blood. The lecithin is believed  
31 effectively to carry the protein with it into general circulation by this means.

32  
33

1 Lecithin may be formed *in vivo* by a variety of different routes. Some of these  
2 are as follows. First, the  $\alpha$ -glycerol pathway may be used;  
3 sn-Glycerol-3-phosphate is used as a precursor in this pathway, as are  
4 phosphatidates and diglycerides. Secondly, lecithin may be synthesised by the  
5 action of cholinephosphotransferase; choline, phosphocholine, cytidine  
6 diphosphocholine and diglycerides are used as precursors in this route. Thirdly,  
7 lecithin may be synthesised from other phosphatides such as phosphatidyl  
8 ethanolamine. Fourthly, lecithin may be synthesised from triglyceride and  
9 indeed lecithin breakdown products or by transesterification processes.

10

11 The term "biologically active material" includes, in particular, pharmaceutically  
12 active proteinaceous materials. The proteinaceous material may be a pure  
13 protein, or it may comprise protein, in the way that a glycoprotein comprises  
14 both protein and sugar residues. The material may be useful in human or  
15 veterinary medicine, either by way of treatment or prophylaxis of diseases or  
16 their symptoms, or may be useful cosmetically or diagnostically. Examples of  
17 proteinaceous biological material which can be provided as orally or rectally  
18 administrable formulations in accordance with this invention include protein  
19 hormones such as insulin, calcitonin and growth hormone, whether from human  
20 or animals or semi- or totally synthetically prepared, erythropoietin or  
21 haematopoietin, plasminogen activators and their precursors, such as t-PA,  
22 urokinase, pro- urokinase and streptokinase, interferons including human  
23 interferon alpha, interferon beta and interferon gamma, interleukins including  
24 IL-1, IL-2, IL-3, IL-4 and IL-5 colony stimulating factors including G-CSF and  
25 GM-CSF and blood factors including Factor VIII.

26

27 It is to be emphasised, however, that the invention is not limited to the  
28 formulation of proteinaceous compounds: many non-proteinaceous  
29 pharmaceutical agents may successfully be formulated by means of the present  
30 invention. For example, non-steroidal anti-inflammatory drugs (NSAIDs) such  
31 as indomethacin and other agents including gentamycin may appropriately be  
32 formulated.

33

1 However, in view of the co-formulation of the biologically active material with,  
2 for example, phospholipids in this invention, it is desirable that the active  
3 material is not one that irreversibly forms a covalent bond with a phospholipid,  
4 or indeed any of the other components of the formulation, as this may in some  
5 circumstances impair biological activity and/or availability. Having said that, it  
6 is not believed that there is any problem on this account with the formulation by  
7 means of the invention of any of the active molecules specified above. The  
8 association between the active compound and the lecithin or precursor may be in  
9 the nature of a non-covalent complex. Such a complex may involve hydrogen  
10 bonding, van de Waals interactions, ionic interactions and/or lipid-lipid  
11 interactions.

12  
13 While it is not believed that there is any particular molecular size constraint on  
14 biologically active materials that can be formulated by means of the present  
15 invention, it will be apparent from the exemplary but non-limiting selection of  
16 biologically active materials given above that the invention is particularly  
17 suitable for formulating macromolecules. The molecular weight of such  
18 macromolecules may be about 1kDa or above 5kDa, about 10kDa or above, or  
19 even about 15kDa or above. Again, while it is not believed that hydrophilicity  
20 or hydrophobicity (lipophilicity) of the biologically active material is  
21 particularly critical, the invention readily enables the formulation of hydrophilic  
22 molecules such as insulin, calcitonin (especially salmon calcitonin) and growth  
23 hormones or somatotropin (especially porcine somatotropin), all of which  
24 (particularly salmon calcitonin) are so hydrophilic as to be hygroscopic.

25  
26 The amount of biologically active material present in a formulation of the  
27 invention will naturally depend on the nature of the material and will be such an  
28 amount as to make prescription of conveniently administrable amounts a  
29 practicable proposition. Bearing these considerations in mind, formulations in  
30 accordance with the invention may contain from 1  $\mu$ g, 10  $\mu$ g, 0.1 mg or 1 mg  
31 per litre to 1, 10g or 100g per litre.

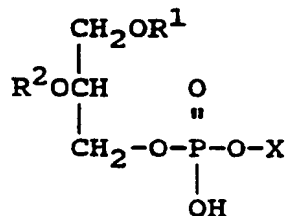
32  
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1 The present invention involves derivatives or constituent parts or groups of  
 2 phospholipids or other compounds which are capable of acting as precursors for  
 3 the *in vivo* synthesis of lecithin at the human or other animal intestinal  
 4 epithelium; the lecithin in turn forms at least part of the membrane to the  
 5 chylomicron core. It is believed that under the conditions of administration, the  
 6 membrane-integrating compounds cause the associated biologically active  
 7 material to be integrated into a lecithin membrane covering for example a  
 8 chylomicron core; the membrane is composed primarily of phospholipid, as  
 9 discussed above. Because chylomicron membranes are phospholipid-rich,  
 10 phospholipids are very suitable materials with which to formulate biologically  
 11 active materials in accordance with the invention.

12  
 13 The lecithin precursors should not be such as to cause deterioration of the  
 14 biologically active material; for example, it has been reported that some fatty  
 15 acids, such as oleate and stearate, may interact adversely with porcine  
 16 somatotropin, so a certain but routine amount of care should be used when  
 17 selecting the phospholipids, phospholipid derivatives or other  
 18 membrane-integrators or precursors to be used. The selection will however be  
 19 well within the capabilities of those skilled in the art.

20  
 21 Phospholipids are the lecithin precursors of choice. Phospholipids are glyceryl  
 22 triesters in which one of the ester functions is an optionally substituted  
 23 phosphoric acid. Phospholipids preferred for use in the present invention have  
 24 the following general formula:



1 wherein each of R<sup>1</sup> and R<sup>2</sup> independently represents an acyl group of for  
2 example 10, 12 or 14 to 26 carbon atoms which is optionally mono- or  
3 poly-unsaturated and X represents a hydrogen atom or a phospholipid head  
4 group.

5  
6 The phospholipid head group may be any group that is capable of forming a  
7 physiologically acceptable phospholipid. Examples of phospholipids include:

8  
9 diacyl phosphatidyl glycerols, such as:

10

11 dimyristoyl phosphatidyl glycerol (DPMG),  
12 dipalmitoyl phosphatidyl glycerol (DPPG), and  
13 distearoyl phosphatidyl glycerol (DSPG);

14

15 diacyl phosphatidyl cholines, such as:

16

17 dimyristoyl phosphatidylcholine (DPMC),  
18 dipalmitoyl phosphatidylcholine (DPPC), and  
19 distearoyl phosphatidylcholine (DSPC);

20

21 diacyl phosphatidic acids, such as:

22

23 dimyristoyl phosphatidic acid (DPMA),  
24 dipalmitoyl phosphatidic acid (DPPA), and  
25 distearoyl phosphatidic acid (DSPA); and

26

27 diacyl phosphatidyl ethanolamines such as:

28

29 dimyristoyl phosphatidyl ethanolamine (DPME),  
30 dipalmitoyl phosphatidyl ethanolamine (DPPE), and  
31 distearoyl phosphatidyl ethanolamine (DSPE).

32

33

1 Other examples include, but are not limited to, derivatives of ethanolamine  
2 (such as phosphatidyl ethanolamine, as mentioned above, or cephalin), serine  
3 (such as phosphatidyl serine) and 3'-O-lysyl glycerol (such as  
4 3'-O-lysyl-phosphatidylglycerol).

5  
6 More than one phosphatidyl group may be attached to a single phospholipid  
7 head group; for example, two phosphatidyl moieties may be attached to a single  
8 glycerol residue as in diphosphatidyl glycerol or cardiolipin. When X represents  
9 a hydrogen atom, the phospholipid is a phosphatidic acid such as  
10 L- $\alpha$ -phosphatidic acid bimyristoyl.

11  
12 Phospholipids useful in the present invention include synthetic and natural  
13 phospholipids, whether as single components or as a mixture of two or more  
14 components. Preparations of ostensibly pure natural phospholipids will rarely if  
15 ever actually contain a single species of phospholipid, but this factor is not  
16 believed to be critical for the purposes of the present invention.

17  
18 Particularly preferred phospholipids include  
19 1,2-dimyristoyl-*sn*-glycerol-3-phosphocholine, which may be in the form of the  
20 monohydrate and L- $\alpha$ -phosphatidic acid bimyristoyl, which may be in the form  
21 of the sodium salt.

22  
23 Other precursors of lecithin may be used instead or in addition.

24  
25 In compositions of this invention, the biologically active material may be in  
26 association with the lecithin precursor. While the precise nature of this  
27 association is not necessarily critical, it is believed that it may involve  
28 non-covalent interactions, particularly hydrogen bonding and hydrophobic  
29 interaction, much in the same way that lipoproteins conventionally present in  
30 chylomicron or other phospholipid membranes are bound.

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33

1 In the presence of one or more high hydrophile/lipophile balance (HLB)  
2 surfactants, such as those having an HLB value above 10 or even above 14, the  
3 biologically active material, in association with the lecithin precursor, may form  
4 a hydrophilic complex which passes readily in to the enterocytes (gut epithelial  
5 wall cells). Within the enterocytes, a lecithin precursor is recognised as such,  
6 for use in lecithin synthesis. In this way, the precursor and the associated  
7 biologically active material appears to avoid the lysosome and is converted into  
8 a complex of biologically active material and membrane integrating compound  
9 (such as lecithin). Such a complex may replace or supplement the lecithin which  
10 forms the outer-layer membrane covering about 80% or more of the surface of  
11 the chylomicron core.

12  
13 In the circulating blood, surface proteins and phospholipids may exchange with  
14 other lipo-proteins. So at least a portion of a protein administered by means of  
15 the present invention may circulate in the blood as a phospholipoprotein  
16 separated from a chylomicron with which it was originally or previously  
17 associated. Some of the phospholipoprotein may be released into the circulating  
18 blood in free form and, in part, may be passed to the liver attached to  
19 chylomicron remnants. The speed and extent of the phospholipid/protein  
20 exchange may be influenced by various factors, including altering the  
21 phospholipid chain length.

22  
23 Formulations in accordance with the invention may generally also contain a  
24 hydrophilic liquid, which will usually be aqueous and may be water;  
25 physiological or phosphate-buffered saline may satisfactorily be used. A water  
26 miscible solvent may be present, for example to aid in formulating. Ethanol or  
27 another suitable simple organic solvent may therefore be present. The nature of  
28 the solvent used will depend on the active material. The hydrophilic liquid may  
29 be as water:solvent mix, for example in v/v proportions of 0.5:1 to 2:1,  
30 although the presence of a non-aqueous solvent is not necessarily preferred.

31  
32  
33

1 Broad and preferred percentage compositions (which will generally be  
2 weight/weight percentages, but may be weight/volume or even volume/volume  
3 percentages) of components are given below, providing always that the total  
4 does not exceed 100%:

5			
6		<u>Broad</u>	<u>Preferred</u>
7	Precursor/active	0.1 - 25	1 - 10
8	Hydrophilic liquid	10 - 99	50 - 95
9			
10		<u>More Preferred</u>	<u>Optimal</u>
11	Precursor/active	2.5 - 8	4
12	Hydrophilic liquid	65 - 90	89

13  
14 Formulations in accordance with the invention may contain a hydrophilic  
15 surfactant (for example with an HLB greater than 10). This may have the effect  
16 of promoting the formation of a complex between the biologically active  
17 compound and the lecithin precursor (particularly for synthesised lecithin or  
18 lecithin precursors in the gut epithelium in humans and certain other animals  
19 such as pigs), and/or of conferring a broadly hydrophilic character on such a  
20 complex. The hydrophilic surfactant may be present in an amount up to 10%  
21 (w/v or v/v), preferably from 1 to 5%, typically from 1.5 to 4%, for example  
22 about 2.4% or 2.5%.

23  
24 One further component that is often highly desirable is a protease inhibitor,  
25 which may be in the form of one or more individual protease inhibitors.  
26 Protease inhibitors useful for the present invention can broadly be divided into  
27 two categories. First, there is the category of protease inhibitors which are  
28 useful in limiting or preventing the degradation of the biologically active  
29 material if it is proteinaceous. Such protease inhibitors should have the effect of  
30 inhibiting proteolytic enzymes found in the gastrointestinal tract, such as  
31 trypsin, chymotrypsin and carboxypeptidase. In the case of insulin, the protease  
32 inhibitors will generally be inhibitory of the class of enzymes that have come to  
33 be known as insulinase, which includes the enzyme trans-sulphatase. Suitable

1 sources of trypsin inhibitors can be extracted from soy beans or egg white  
2 (ovomucoid). Secondly, if apoprotein is present in formulations in accordance  
3 with the invention, it is desirable to add protease inhibitors to reduce the amount  
4 of degradation of the apoprotein before it reaches the intestinal mucosa.  
5 Generally speaking, similar protease inhibitors can be used as for the protection  
6 of proteinaceous biologically active materials, and so a single protease inhibitor  
7 may serve both functions. Protease inhibitors may be added to the association  
8 or complex between the biologically active material and the membrane  
9 integrator or precursor (for example the phospholipids); for convenience they  
10 may be added to the hydrophilic phase, where two phases are present. The  
11 choice of the amount of protease inhibitor to be added will be well within the  
12 skill of a person skilled in the art, but generally will be in amounts up to about  
13 0.1% w/v, or even 0.5% w/v. Aprotinin may be added in an amount up to 10  
14 million IU, preferably 0.5 to 5 million IU, typically 1.5 to 4 million IU, for  
15 example 3.0 million IU, but the exact amount used may depend on the actual  
16 activity of the biologically active material.

17  
18 It will in many cases be advantageous to administer the complex of biologically  
19 active material and membrane integrator or precursor (the phospholipoprotein  
20 complex in the preferred embodiment), together with the optional but preferred  
21 hydrophilic surfactant and protease inhibitor, suspended in, or made into an  
22 emulsion or microemulsion containing lipophilic material including a low HLB  
23 surfactant (for example having an HLB value of less than 4). The lipophilic  
24 material may (but does not necessarily) include those material known to form  
25 chylomicra *in vivo*; such material includes but is not limited to cholesterol,  
26 cholesterol ester(s), lecithin and/or other phospholipids or saturated or mono- or  
27 polyunsaturated fatty acids (for example having a carbon content of  $C_{16}$  to  $C_{24}$ ),  
28 which may optionally be esterified as a glycerol ester to form a mono-, di- or  
29 triglyceride. Alternatively, the essentially hydrophilic phospholipo- protein (or  
30 other complex) may simply be mixed with suitable oils, particularly vegetable  
31 oils, such as medium chain triglyceride (MCT) oil or any other appropriate oil,

32  
33

1 plus one or more suitable surfactants having a low HLB value (for example less  
2 than 4). Suitable surfactants include lysolecithin derivatives and other essentially  
3 lipophilic materials.

4  
5 The hydrophilic phospholipoprotein (or other complex) may be appropriately  
6 enteric coated and may be orally administered. However, experiments suggest  
7 that it is preferable to mix the complex with a suitable oil or precursors so as to  
8 channel the active material into the villae of the small intestine tract from where  
9 it is absorbed through the villae and drained into the lymphatic system.

10  
11 Broad and preferred percentage compositions (which will generally be  
12 weight/weight percentages, but may be weight/volume or even volume/volume  
13 percentages) of the lipophilic material for general purposes are given below,  
14 providing always that the total does not exceed 100%:

	<u>Broad</u>	<u>Preferred</u>
16 Cholesterol	0.1 - 40	1 - 10
17 Lecithin	0.1 - 60	1 - 15
18 (or other phospholipid)		
19 Lipophilic surfactant	0.1 - 40	3 - 10
20 Non-esterified fatty acid	0 - 95	20 - 90
21 Cholesterol ester	0 - 10	0 - 5

	<u>More preferred</u>	<u>Optimal</u>
24 Cholesterol	2 - 8	6
25 Lecithin	4 - 10	8
26 (or other phospholipid)		
27 Lipophilic surfactant	4 - 8	6
28 Non-esterified fatty acid	35 - 75	50

1 Some lipophilic-phase miscible organic solvent may be present, possibly as an  
2 aid in formulation. The nature of the solvent will depend on the other materials  
3 present. Ethanol is often suitable. The amount of solvent may be, for example  
4 from 5 to 50% v/v, based on the volume of the lipophilic phase.

5  
6 When the phospholipoprotein or other complex is formulated as an emulsion or  
7 microemulsion with a lipophilic phase as discussed above (usually as a  
8 water-in-oil system) it is not essential for any other ingredients to be present  
9 although, as a matter of practice, it is usually highly convenient for other  
10 ingredients to be added. An optional ingredient is a stabiliser for the biologically  
11 active material. The precise nature of the stabiliser, if present, will of course  
12 depend on the nature of the biologically active material itself. For example,  
13 there are a number of well defined stabilisers for insulin, which can be  
14 advantageously be incorporated in insulin-containing formulations in accordance  
15 with the invention. Examples include hydroxypropyl cellulose (HPC), calcium  
16 salts and citric acid and its salts. Calcium is known not only to stabilise insulin  
17 but also to have an additional beneficial effect of increasing the porosity of cell  
18 membranes, thereby facilitating entry of the active material into the intestinal  
19 wall cells. As the biologically active material is added in the hydrophilic  
20 phase, the stabiliser will for preference normally be added in that phase too.  
21 The amount of stabiliser to be present will again depend on its nature and the  
22 nature of the biologically active material; the choice of the amount will be well  
23 within the capabilities of a person skilled in the art but will often be in amounts  
24 up to about 1 or 2% w/v.

25  
26 It may be desirable in some instances to incorporate emulsification aids, which  
27 may be conventional emulsification aids used in the preparation of emulsions.  
28 Some emulsification aids are surfactants, and surfactants useful for this purpose  
29 are not restricted to any particular HLB values. Useful emulsification aids  
30 include cholesterol, stearic acid, sodium stearate, palmitic acid, sodium  
31 palmitate, oleic acid, sodium oleate, glyceryl monooleate, polyoxyethylene 50

32  
33



1    stearate, polyoxyethylene 40 stearate, polysorbate 20, polysorbate 40,  
2    polysorbate 60, polysorbate 80, propylene glycol diacetate, and propylene  
3    glycol monostearate.

4  
5    Emulsification aids may be present in either or both of the lipophilic and  
6    hydrophilic phases. The amount of emulsification aid to be present, if desired,  
7    will simply be enough to assist in adequately obtaining a stable formulation.  
8    The exact amount can be determined by a person skilled in the art; generally  
9    speaking they can be used in amounts of from 0 to 15% w/v, for example 0.1 to  
10   5% w/v of the formulation as a whole. it may be appropriate to provide, say  
11   from 1 to 5% in the hydrophilic phase of the same or a different surfactant. It  
12   has been found to be particularly appropriate to add polysorbate 80 to the  
13   lipophilic phase and polyoxyethylene 40 stearate to the hydrophilic phase.

14  
15   Formulations in accordance with the invention can contain various  
16   preservatives. Two particularly useful categories of preservatives are  
17   antioxidants and antimicrobial agents. Antioxidants are particularly useful  
18   because certain compounds suitable for use in formulations of the invention are  
19   prone to degradation by autoxidation. Although this problem can be avoided by  
20   preparing formulations in accordance with the present invention under an inert  
21   atmosphere, such as nitrogen, this is a somewhat inconvenient and expensive  
22   process and so it is often preferred to add chemical anti-oxidants. Suitable  
23   pharmaceutically acceptable antioxidants include propyl gallate, butylated  
24   hydroxyanisole, butylated hydroxytoluene, ascorbic acid or sodium ascorbate,  
25   DL- or D- $\alpha$ -tocopherol and DL- or D- $\alpha$ -tocopheryl acetate. The anti-oxidant, if  
26   present, may be added to formulations in accordance with the invention in an  
27   amount of up to, for example, 0.1% (w/v), preferably from 0.0001 to 0.3%.  
28   The appropriate phase for the antioxidant will naturally depend on the nature of  
29   the antioxidant. Generally lipophilic antioxidants such as  $\alpha$ -tocopherol may  
30   appropriately be incorporated into the hydrophobic phase, whereas hydrophilic  
31   antioxidants such as ascorbic acid may be incorporated into the hydrophilic  
32   phase.

33

1 Sesame oil, preferably as a refined chemical oil, may be added to formulations  
2 of the invention, as it has anti-oxidant activity. Sesame oil has the further  
3 advantage that it improves the flavour of the formulations, thereby improving  
4 patient compliance. Sesame oil may be present in an amount of from 0.1 to 3%  
5 w/v preferably 5 to 20% w/v of the final liquid formulation; it will usually be  
6 added to the lipophilic phase.

7  
8 Antimicrobial preservatives which may be used, generally in amounts of up to  
9 about 3% w/v, preferably from about 0.5 to 2.5%, of the total formulation,  
10 include methylparaben, ethylparaben, propylparaben, butylparaben, phenol,  
11 dehydroacetic acid, phenylethyl alcohol, sodium benzoate, sorbic acid, thymol,  
12 thimerosal, sodium dehydroacetate, benzyl alcohol, cresol, p-chloro-m-cresol,  
13 chlorobutanol, phenylmercuric acetate, phenylmercuric borate, phenylmercuric  
14 nitrate and benzylalkonium chloride. Antimicrobial agents can be added to  
15 either phase as required or appropriate.

16  
17 Although not essential, it may be practical or convenient to improve  
18 trans-lymphatic absorption of the phospholipoprotein or other complexes in  
19 humans and certain other species when formulations of the invention are in  
20 two-phase form. Two-phase systems in accordance with the invention include  
21 water-in-oil (ie hydrophilic-in-lipophilic), water-in-oil-in-water, oil-in-water and  
22 oil-in-water-in-oil systems.

23  
24 Two-phase systems can in general be prepared by intimate admixture of the  
25 hydrophilic and lipophilic phases. Two-phase systems in accordance with the  
26 invention may be emulsions or microemulsions. The volume:volume ratio of the  
27 hydrophilic phase:lipophilic phase will generally be in the range of from 0.2:1  
28 to 5:1, typically from 0.5:1 to 2:1.

29  
30 To form emulsions or microemulsions, it is sometimes necessary to use two  
31 different surfactants, one being hydrophilic and having a high  
32 hydrophile-lipophile balance (HLB), and the other being more lipophilic (as  
33 described above), and having a low HLB. Hydrophilic surfactants useful in the

1 present invention, when present, have a high HLB of at least 10 or a very high  
2 HLB of at least 17 and possibly approaching 20. Lipophilic surfactants used in  
3 the invention have a low HLB of, for example, less than 10. Preferably, the  
4 lipophilic surfactant has an HLB value of less than 7 or even less than 4.

5  
6 As general guidance it is preferred that each of the surfactants used in the  
7 preparation of formulations of this invention be selected from those surfactants  
8 classified as anionic or nonionic. These surfactants are particularly useful in  
9 pharmaceutical systems for their compatibility, stability, and non-toxicity.  
10 Surfactants generally suitable for the various purposes in the present invention  
11 include:

- 12
- 13 - long chain ( $C_{16}$  to  $C_{24}$ ) fatty acids, e.g. palmitic acid, stearic acid and  
14 oleic acid;
  - 15
  - 16 - esters of long chain ( $C_{16}$  to  $C_{24}$ ) fatty acids, e.g. sodium palmitate,  
17 sodium stearate and sodium oleate;
  - 18
  - 19 - sodium lauryl sulphate;
  - 20
  - 21 - fatty acid esters of polyethylene glycol, e.g. polyethylene glycol mono-  
22 or di-stearate;
  - 23
  - 24 - propylene glycol and fatty acid esters of propylene glycol, e.g.  
25 propylene glycol monostearate;
  - 26
  - 27 - glycerine and fatty acid mono- or poly-glycerides, such as glyceryl  
28 monostearate;
  - 29
  - 30 - polyoxyethylene fatty acid esters, ethers and amines, e.g.  
31 polyoxyethylene mono- and di-stearate, and polyoxyethylene lauryl  
32 ether;
  - 33

- 1 - polyoxyethylene sorbitan esters, e.g. polyoxyethylene sorbitan  
2 monolaurate, monopalmitate, monostearate or mono-oleate;  
3
- 4 - polyoxyethylene alkyl phenols and alkyl phenyl ethers;  
5
- 6 - polyoxyethylene castor oil;  
7
- 8 - sorbitan fatty acid esters;  
9
- 10 - the polysorbates; stearylamine; triethanolamine oleate;  
11
- 12 - vegetable oils, e.g. sesame seed oil or corn oil;  
13
- 14 - cholesterol; and  
15
- 16 - tragacanth.  
17

18 The surfactants of choice will of course be those which are currently on the  
19 approved list for pharmaceutical use and will have appropriately low LD<sub>50</sub>  
20 values. There follows a list of certain exemplary surfactants, together with their  
21 HLB values and, where known, their LD<sub>50</sub> values.  
22

23 Examples of suitable high HLB surfactants are as follows:  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33

1			
2			
3	Chemical Identity	HLB	LD <sub>50</sub> g/kg
4			
5	<u>Polyethylene Glycol Esters</u>		
6			
7	PEG-monostearate	19.1	?
8			
9	<u>Polyoxyethylated Glycol Monoethers</u>		
10			
11	POE(23) lauryl ether	17.0	9
12			
13	<u>Polyoxyethylated Fatty Acids</u>		
14			
15	POE(40) lauric acid	17.9	?
16	POE(100) lauric acid	19.1	?
17	POE(40) oleic acid	17.4	?
18	POE(100) oleic acid	18.8	?
19	POE(40) stearic acid	17.8	?
20	POE(50) stearic acid	17.9	>25
21	POE(100) stearic acid	18.8	25
22			
23			
24			
25			
26			
27			
28			
29			
30			
31			
32			
33			

1 Examples of suitable low HLB surfactants are as follows:

4	Chemical Identity		HLB	LD <sub>50</sub> g/kg
5				
6	<u>Glycerol Esters</u>			
7				
8	Glycerol monooleate		3.8	?
9				
10	<u>Polyoxyethylated Glycol Monoethers</u>			
11				
12	POE(4)	lauryl ether	9.5	9
13	POE(2)	cetyl ether	5.3	22
14	POE(2)	stearyl ether	4.9	>25
15	POE(2)	oleyl ether	4.9	25
16				
17	<u>Polyoxyethylated Fatty Acids</u>			
18				
19	POE(4)	lauric acid	9.3	?
20	POE(4)	oleic acid	7.7	?
21	POE(4)	stearic acid	7.7	?
22				
23	<u>Sorbitan Fatty Acid Esters</u>			
24	Sorbitan monolaurate		8.6	41
25	Sorbitan monopalmitate		6.7	>16
26	Sorbitan monostearate		4.7	31
27	Sorbitan tristearate		2.1	>16
28	Sorbitan monooleate		4.3	>40
29	Sorbitan sesquioleate		3.7	?
30	Sorbitan trioleate		1.8	>40
31	Sorbitan monoisostearate		4.7	?
32				
33				

1 Polyoxyethylated Sorbitan Fatty Esters

2

3 POE(4) sorbitan monostearate | 9.6 &gt;40

4 POE(5) sorbitan monooleate | 10.0 &gt;37

5

6 Polyoxyethylated Castor Oils

7

8 POE(10) castor oil | 6.3 ?

9 POE(10) hydrogenated castor oil | 6.3 ?

10

11 Poloxamers

12

13 POE(7) - POP (17) (L42) | 8 ?

14 POE(4) - POP (23) (L61) | 3 ?

15 POE(10) - POP (23) (L62) | 7 ?

16 POE(27) - POP (23) (L64) | 7 ?

17 POE(6) - POP (30) (L81) | 2 ?

18 POE(19) - POP (37) (L92) | 5.5 ?

19 POE(8) - POP (43) (L101) | 1 ?

20 POE(32) - POP (43) (P103) | 9 ?

21 POE(10) - POP (53) (L121) | 0.5 ?

22

23 It should be noted that mixtures of surfactants can often be used in place of  
 24 single surfactants in the present invention. For example, instead of a single  
 25 hydrophilic surfactant, a mixture of two or more relatively hydrophilic  
 26 surfactants could be used; the effective HLB of the mixture should, however, be  
 27 greater than 10. By "effective HLB" is meant that the hydrophile-lipophile  
 28 balance of the mixture of surfactants should be equivalent to a single surfactant  
 29 having an HLB of greater than 10. Similarly, mixtures of lipophilic surfactants  
 30 can be used in place of a single lipophilic surfactant. Again, the effective HLB  
 31 of the lipophilic surfactants should be less than 10.

32

33

1 The choice of the amount of surfactant to be used in formulations of the present  
2 invention is left as a matter of choice to those skilled in the art. Naturally,  
3 precise amounts that will be optimal in each case will depend very much on the  
4 precise nature of the surfactants used and what other ingredients in the  
5 formulations are present. Nevertheless, as general guidance, the amount of  
6 hydrophilic surfactant, when present, will generally be in the range (based on  
7 the total volume of the formulation) of from 0.1 g to 50 g per litre, with a range  
8 of from 0.5 to 25 g per litre usually being preferred and from 1 g to 10 g per  
9 litre often being optimal. The lipophilic surfactant has been discussed above in  
10 relation to the oil phase of the microemulsion. It will generally be present in an  
11 amount of from 0.1 g to 100 g per litre, with a range of from 0.5 g to 50 g per  
12 litre being preferred and a range of from 2 g to 25 g per litre often being  
13 optimal, with the figures again being based on the total volume of the  
14 formulation.

15  
16 Compositions in accordance with the invention may be prepared, most broadly,  
17 by admixture of the ingredients. According to a second aspect of the invention,  
18 there is therefore provided a process for the preparation of a composition as  
19 described above, the process comprising admixing the ingredients of the  
20 composition.

21  
22 It is generally preferred for the active (usually proteinaceous) compound and the  
23 lecithin precursor to be admixed first. This enables the "phospholipo-protein"  
24 complex to be formed in preferred embodiments.

25  
26 As discussed above, some compositions in accordance with the invention  
27 involve two phases. A preferred process for preparing such compositions  
28 comprises:

29  
30 (i) providing a hydrophilic phase comprising a biologically active  
31 substance and a lecithin precursor; and  
32  
33



1 (ii) forming a two-phase system including the hydrophilic phase,  
2 optionally in conjunction with one or more absorbtion enhancers.  
3

4 Because of the inherent thermodynamic stability of certain emulsions and  
5 microemulsions, liquid formulations in accordance with the invention can  
6 simply be prepared by mixing the hydrophilic and lipophilic phases, which in  
7 turn can be prepared by mixing their respective ingredients together. Kinetic  
8 considerations, however, suggest that as a practical matter certain steps be taken  
9 to ensure the rapid and effective formation of emulsion or microemulsion  
10 formulations in accordance with the invention. In particular, during or after the  
11 hydrophilic and lipophilic phases have been added together, a microemulsion  
12 can be speedily formed by the use of a microfluidiser, and an emulsion can be  
13 prepared by using an appropriate apparatus which gives intimate admixture.  
14

15 It will be appreciated that some formulations in accordance with the invention,  
16 particularly two-phase formulations, are likely to be liquid. However, they can  
17 be converted into solid, powdery forms by conventional methods. In general,  
18 the liquid form may be coated onto solid carrier powders by using a fluidiser  
19 bed or similar equipment (such as a SPIR-A-FLOW apparatus). (The expression  
20 SPIR-A-FLOW is a trade mark.) Powder or granules resulting from this  
21 operation may be packed into hard gelatin capsules, which may then be enteric  
22 coated if desired. Alternatively, the resulting powder or granules may be made  
23 into granules sized about 1 to 2 mm, which can then be enteric coated and  
24 placed into hard gelatin capsules. Alternatively, the liquid formulation may be  
25 packaged into soft gelatin capsules, which, if required and feasible, can be  
26 enteric coated. Suitable enteric coating materials are known, for example, from  
27 "Remington's Pharmaceutical Sciences", 15th Edition, pp. 1614-1615 (1975);  
28 2nd Edition, pp 116-117, 371-374 (1976); and "Hagers Handbuch der  
29 Pharmazeutischen Praxie", 4th Edition, Volume 7a (Springer Verlag 1971),  
30 pages 739 to 742 and 776 to 778.  
31  
32  
33

1 Formulations in accordance with the invention can therefore be administered  
2 orally, but in a wide variety of different ways, for example as a liquid, as a soft  
3 gelatin capsule, as a hard gelatin capsule, as a pressed tablet (which may also be  
4 enteric coated) and in other ways. Furthermore, high plasma levels as well as  
5 high levels at the supposed target receptors indicate that biologically active  
6 materials administered by means of the invention have high bioavailability and  
7 that the active material is bioactive.

8  
9 For rectal administration, liquid or solid formulations can be administered as an  
10 enema or in suppository form. The suppository base may be cocoa butter or  
11 any other suitable material.

12  
13 According to a further aspect of the invention, there is therefore provided a  
14 method of treating a human or other animal, comprising the oral or rectal  
15 administration of a formulation in accordance with the first aspect of the  
16 invention. In particular, the invention extends to the treatment of diabetes by  
17 the rectal or preferably oral administration of a formulation in accordance with  
18 the invention in which the biologically active material is insulin.

19  
20 The invention also extends to the use of the ingredients of formulations in  
21 accordance with the first aspect of the invention in the preparation of an orally  
22 or rectally administrable formulation for the treatment or prophylaxis of  
23 disorders treatable or controllable by a biologically active material.

24  
25 In particular, insulin can be used in the preparation of a formulation for the  
26 treatment or control of diabetes. Salmon calcitonin can be used in the treatment  
27 of high bone turnover (for example in Paget's disease of the bone), acute  
28 hypercalcaemia associated with malignancy and osteoporosis. Erythropoietin can  
29 be used in the treatment of anaemia arising from chronic usage of either  
30 extracorporeal renal dialysis devices or anti-cancer chemotherapeutics or other  
31 causes. Porcine somatotropin can be administered to pigs to reduce the raising  
32 time of pigs and possibly to reduce the thickness of back fat. Human growth  
33 hormone can be used to treat children with a retarded growth rate.

1 The invention will now be illustrated by a number of non-limiting examples.

2

3 EXAMPLE 1

4

5 In this example, insulin is the biologically active ingredient and a formulation  
6 containing a phospholipo-insulin complex is prepared.

7

8 At room temperature, bovine insulin (2 to 20mg of bovine insulin crystalline or  
9 powder having about 22 to 26 IU activity per mg) is added 1.0 to 3.0 g of  
10 soybean lecithin, egg yolk lecithin, L- $\alpha$ -phosphatidic acid bimyristoyl (sodium  
11 salt), and/or 1,2-dimyristoyl-sn- glycerol-3-phosphocholine monohydrate and  
12 dissolved in either 0.9% benzyl alcohol or 0.9% sodium chloride (150ml) in  
13 presence of 400mg of aprotinin (@3,000,000 Kallikrein inactivator units),  
14 adjusted to pH @ 2.3 with citric acid solution in room temperature. A  
15 non-ionic surfactant, preferably polyoxy-40-stearate (4 g) is added to the above  
16 'phospholipid-insulin' compound.

17

18 Under gentle and constant stirring at room temperature, the above  
19 phospholipo-insulin solution is slowly added into an oil-phase solution  
20 comprising pre-chylomicron complexes (which are complexes of  
21 phosphatidylcholine; mono-, di-, and/or tri-glyceride; cholesterol and others), a  
22 non-ionic surfactant with an HLB value less than 4.0 and one or more  
23 anti-oxidants.

24

25 The above "water-in-oil" emulsion is passed through a microfluidizer at 5 to  
26 10°C, at 100,000 PSI or more for two consecutive times. Thus a  
27 "water-in-oil" microemulsion form of the phospholipo-insulin complex was  
28 prepared. Each 400ml of this microemulsion contains the following ingredients:

29

30

31

32

33

<u>Chemical Composition</u>	<u>per 400ml Microemulsion</u>
Bovine Insulin	87,000 IU
Dimyristoyl-glycerol-phosphocholine*	1.0 g
Aprotinin	2,000,000 KIU
Polyoxy-40-stearate	2.9 g
Cholesterol	11.6 g
Glycerolmonooleate	10.6 g
Oleate	92.5 g
Polysorbate 80	6.8 g
d-alpha-tochopherol	1.2 g
Citric acid	0.9 g
Physiological saline solution	To 400 ml

## EXAMPLE 2

In this example, the biologically active compound is erythropoietin (EPO) and a phospholipo-erythropoietin complex is prepared as in Example 1 above. Also, one-half of EPO was directly added into the water-phase of the two-phase (microemulsion) system. Thus, in this example, the proteinaceous compound was not only bound with phospholipids, but also added directly into the water-phase of microemulsion system.

Erythropoietin (EPO) was supplied by the Chugai Pharmaceutical Company, Ltd. of Tokyo, Japan (Lot No. R9H05). One aliquot had a protein concentration of 0.936 as measured by amino acid analysis, and 1.018 mg as measured by RP-HPLC analysis, and had an *in vivo* specific activity of 180,000 IU in pH7.2 phosphate buffer solution). At room temperature, the EPO aliquot is divided into two halves. To the first half of the EPO aliquot, from 0.004 to 0.007 mg 1,2- dimyristoyl-sn-glycerol-phosphocholine monohydrate per 1000 IU EPO was added and the resulting mixture dissolved in 50 ml of 0.9% physiological saline solution; the pH was adjusted to 7.3 with 0.1M phosphate buffer (pH7.8) in presence of 3000 to 4000 IU of aprotinin (per 1000-15000 IU EPO). The

remaining half of EPO was dissolved in 100 ml of physiological saline solution, adjusted pH to 7.3 as above, and then aprotinin (as above) and a non-ionic surfactant having an HLB value above 7.0, such as polyoxy-40-stearate, were added at concentrations of 0.0044 to 0.00044 mg per 1000 IU EPO; emulsion-stabilisers and viscosity increasers, such as hydroxypropyl cellulose-SL, may be dissolved into the above solution at room temperature.

Under gentle and constant stirring at room temperature, the phospholipo-EPO complex and the EPO-containing 'water-phase solution' is slowly added into an oil-phase solution containing cholesterol, lecithin, glycerol monooleate (a non-ionic surfactant having HLB value of less than 4.0) and anti-oxidants.

The above water-in-oil emulsion was passed through a microfluidizer-emulsifier once in the cold. For each 1000 IU of EPO, the resulting W/O EPO microemulsion may contain the following:

<u>Chemicals</u>	<u>mg/1000IU EPO</u>
EPO (Lot No. R9H05)	1000 IU
1,2,-Dimyristoyl-sn-glycerol- phosphocholine monohydrate	0.0056
Hydroxypropylcellulose-SL	0.880
Polyoxy-40-Stearate	0.440
Aprotinin	3000 KIU
Cholesterol	1.880
Lecithin, Egg Yolk	3.800
Glycerolmonooleate	1.680
d-alpha-Tocopherol	1.180
oleic acid	15.000
Tween-80	1.06
Ethanol	7.00*

(\*To be evaporated in most part).

1 EXAMPLE 3

2

3 In this example, a phospholipo-porcine somatotropin (pST) complex and  
4 formulation was prepared according to the present invention.

5

6 Porcine somatotropin (pST) crystalline powder (pST Lot No. 7368C-25Q) was  
7 supplied by American Cyanamid Company, Agricultural Research Division, of  
8 Princeton, New Jersey, USA. At room temperature, pST is added to a 0.9%  
9 saline solution containing phospholipids, such as  
10 1,2-dimyristoyl-sn-glycerol-3-phosphocholine mono- hydrate,  
11 L-alpha-phosphatidic acid bimyristoyl (sodium salt), egg yolk lecithin, and/or  
12 soybean lecithin. A non-ionic surfactant having an HLB value of above 7.0 is  
13 also added in presence of aprotinin, a trypsin-inhibitor, in physiological saline  
14 solution.

15

16 The pH of water-phase solution is adjusted, if necessary, to pH 7.2 with 0.1M  
17 NaCl/10mM sodium phosphate buffer. Under gentle and constant stirring at  
18 room temperature, the above phospholipo-pST solution is slowly added into an  
19 oil-phase solution containing a lecithin-cholesterol-glycerol-monoleate mixture;  
20 the resulting mix is run through a microfluidizer-emulsifier twice in cold. Each  
21 ml of pST microemulsion contains the following:

22

23 Chemicalsmg/ml pST Emulsion

24

25	Lecithin	12.5
26	Cholesterol	30.48
27	Soy Lecithin	187.43
28	1,2-dimyristoyl-sn-glycerol-3-	
29	phosphocholine monohydrate	1.91
30	L-alpha-phosphatidic acid bimyristoyl	
31	sodium salt	0.095
32	Oleic acid	242.29
33	d-alpha-tocopherol	7.62

1	Glycerol-1-monooleate	27.81
2	Hydroxypropylcellulose-L	14.48
3	Polyoxy-40-stearate	7.62
4	Aprotinin	2850 KIU
5	Tween-80	17.91
6	pH adjusted to 7.2 with sodium	
7	phosphate buffer (10mM)	

8

9 EXAMPLE 4

10

11 Recombinant human growth hormone (r-hGH; Batch No. 9-08 P-508-2, 16th  
12 August 1989) was supplied by SmithKlein Beecham of Philadelphia. The r-hGH  
13 was incorporated into a pharmaceutical formulation broadly as in the other  
14 examples. Specifically, r-hGH (500mg powder) was bound and dissolved in  
15 0.9% NaCl solution; a phospholipid derivative in water was added to form a  
16 water-soluble phospholipo-r-hGH-complex. This was then slowly added into the  
17 oil-phase as before.

18

19 The oil-phase consisted of egg yolk lecithin, cholesterol, glycerol-1-monooleate,  
20 d-alpha- tocopherol, an anti-oxidants solution and Tween-80. This  
21 'water-in-oil' emulsion was passed through the microfluidizer-homogeniser once  
22 in the cold. Each ml of r-hGH microemulsion contains the following:

23

24	<u>Chemicals</u>	<u>mg/ml of r-hGH emulsion</u>
25	Lecithin, egg yolk	37.50
26	L-alpha-phosphatidic acid	
27	bimyristoyl	0.095
28	1,2,dimyristoyl-sn-glycerol-	
29	3-phosphocholine monohydrate	1.91
30	Cholesterol	30.48
31	Glycerol-monooleate	27.81
32	Oleic acid	242.30
33	Tween-80	17.91
34	Water	119.05

35

1 The pH was adjusted to 7.2 with 10mM sodium phosphate buffer.

2

3 (Note that the trypsin-inhibitor aprotinin was not added in this example.)

4

5 EXAMPLE 5

6

7 In this example, salmon calcitonin (supplied by the Rorer Central Research of  
8 Hirsham, Pennsylvania, USA; NPD #8906046 NPP # 211) was bound with egg  
9 yolk lecithin in the presence of aprotinin in 0.9% saline solution; the pH was  
10 adjusted to 2.0 with citric acid and ascorbic acid. In this way, a 'water-soluble'  
11 phospholipid-sCT-aprotinin complex was formed and this was made into a  
12 water-in-oil microemulsion as described in Example 1 above. Each portion of  
13 the formulation containing 100 IU of sCT contained the following:

14

15 Chemicals

mg/100 IU of sCT

16

17	Egg yolk lecithin	13.016
18	Aprotinin	1500 IU
19	sCT	100 IU
20	Polyoxy-40-stearate	2.646
21	Hydroxypropylcellulose-SL	5.026
22	Sodium Benzoate	1.587
23	Citric acid	1.720
24	Ascorbic acid	1.244
25	Cholesterol	10.582
26	d-alpha-tocopherol	0.265
27	Glycerolmonooleate	9.418
28	Oleic acid	84.127
29	Tween-80	6.217
30	Propylparaban	0.529
31	Methylparaban	1.852
32	Sesame seed oil (chemical grade)	2.646
33	Anti-oxidants	1.111
34	Ethanol*	41.336
35	Deionised water	66.137

36 (\*Most of the ethanol is evaporated).



1 EXAMPLE 6

2

3 A powdery or granular form of the insulin preparation of Example 1 may be  
4 prepared by spray coating the above microemulsion onto a pharmacologically  
5 inert carrier base, such as carboxymethylcellulose-Ca, gelatin,  
6 hydroxypropylcellulose-L, alginic acid or a mixture of them. The powdery (or  
7 granular) insulin-containing preparation is packed into No. 1 size hard gelatin  
8 capsules to give the following composition:

9

10 <u>Chemicals</u>	11 <u>mg per Capsule (approximate)</u>
12 Bovine insulin	13 @ 4.5 to 6.0 IU
14 Dimyristoyl glycerol 15 phosphocholine*	16 2.2
17 Aprotinin	18 @ 0.14 KIU
19 Polyoxy-40-stearate	20 1.2
21 Cholesterol	22 5.2
23 Oleic acid	24 42.0
25 Glycerolmonooleate	26 4.8
27 Tween-80	28 3.1
29 Vitamin-E	30 0.55
31 Carboxymethylcellulose-Ca	32 90.9 <sup>+</sup>
33 Alginic acid	45.5 <sup>+</sup>
	22.7 <sup>+</sup>
	25.2 <sup>+</sup>

26

27 Each No. 1 Size Hard gelatinous capsule to weigh 250mg.

28 \* 1,2-dimyristoyl-sn-glycerol-3-phosphocholine  
29 monohydrate

30

31 <sup>+</sup> These are practically non-absorbable in men and are  
32 thus pharmacologically inert, inactive materials.

33

**BIOLOGICAL EXAMPLE A**

Using an oral drug delivery system prepared as described in Example 1 (lecithin-bovine insulin-aprotinin), a clinical study was conducted in a group of known diabetics. The formulation of Example 1 was found to be effective in lowering systemic blood sugar as follows:

9	Patient				Blood Sugar (mg%)							Type
10	<u>Code</u>	<u>Sex</u>	<u>Age</u>	<u>Wt*</u>	<u>0**</u>	30	60	90	120	180	240	
11												
12	A	F	45	55	257	190	169	-	214	138	89	NIDDM
13	B	M	50	42	155	110	108	-	132	149	128	IDDM
14	C	M	60	68	175	149	143	-	160	151	130	NIDDM
15	D	F	40	56	184	-	-	130	-	173	-	IDDM
16	E	M	66	61	169	-	141	-	104	-	-	NIDDM
17	F	F	53	65	316	-	206	-	184	-	-	NIDDM
18	G	F	49	45	320	-	275	-	296	-	-	IDDM
19	H	F	57	67	169	-	167	-	121	-	-	NIDDM
20	J	M	43	70	186	-	173	-	137	-	-	IDDM
21	K	M	69	66	195	-	-	136	-	154	-	NIDDM

\* Wt=Body weight (Kg); \*\* 0= 0-Minute

It was interesting to note that, in general, the onset of the hypoglycaemic effect of the lecithin-bovine insulin formulation after its oral administration was rather fast, occurring between 30 to 90 minutes after medication; blood sugar levels were slightly elevated at 120 minutes or so after the oral dosing of insulin in this formulation, and were lowered again after 3 hours or more: this oral formulation of insulin therefore has a dual phasic effect. By binding the lipophilic regions of bovine insulin with phospholipids, such as lecithin, a phospholipo-insulin complex (which then becomes a hydrophilic compound like other lipoproteins are in human body) is formed. Part of the

phospholipo-insulin complex appears to cause an immediate hypoglycaemic reaction by its immediate absorption from the human gastrointestinal system; the rest of it (, that is, that part which did not react with peripheral insulin-receptors) appears to be directed into the liver, stored temporarily in the liver, and then released into the circulating blood causing the second reaction at the insulin receptors.

#### BIOLOGICAL EXAMPLE B

Eight healthy, normal male volunteers participated in this study. After overnight fasting, on Study Day 1 the subjects received medication as follows:

<u>Subject</u>	<u>Study Medication EPO dose (IU)</u>
A	EPO intravenous infusion 10 IU/Kg
B	ODDS-EPO, <u>per os</u> 20 IU/Kg
C	Placebo, <u>per os</u> 0 IU/Kg
D	EPO intravenous infusion 10 IU/Kg
E	ODDS-EPO, <u>per os</u> 15 IU/Kg
F	Placebo, <u>per os</u> 0 IU/Kg
G	ODDS-EPO, <u>per os</u> 20 IU/Kg
H	ODDS-EPO, <u>per os</u> 15 IU/Kg

Blood samples were collected at time 0, 0.5, 1, 2, 3, 4, 6, 10 and 14 hours after the medication; reticulocyte counts (%) were made and plasma EPO levels were measured by radioimmunoassay.

On Day 2 and Day 3, each subject was given the assigned coded study medications at 08:00 a.m., 14:00 p.m. and 22:00 p.m. and, after an overnight fast, on Study Day 4, each subject was examined again after having been given the study medications as above.

1 Reticulocyte counts, especially, on Study Day 4, were increased after 20 IU/kg  
2 and after 15 IU/kg of oral EPO as well as after 10 IU/Kg EPO intravenous  
3 infusion in these normal volunteers. The reticulocyte counts gradually  
4 decreased in the placebo group.

5  
6 On Study Day 1, plasma RIA-measured EPO levels were markedly increased  
7 after both 20 IU/kg and 15 IU/kg oral EPO as well as after 10 IU/kg EPO,  
8 intravenous infusion. With the placebo group, once again the EPO levels  
9 gradually decreased over the study period. EPO delivered by the formulation of  
10 Example 2 was orally effective and bioavailable in men.

11  
12 BIOLOGICAL EXAMPLE C

13  
14 The formulation prepared in Example 3 (a  
15 dimyristoylglycerol-phosphocholine-phosphatidic bimyristoyl-pSCT complex),  
16 was intraduodenally infused into a group of conscious pigs. Serial blood sugar  
17 levels and plasma pST levels (measured by radioimmunoassay) were assayed  
18 before and after the administration of the formulation of Example 3. The  
19 results, below, show a correlative rise in blood sugar and an increase in  
20 RIA-measured pST levels.

21  
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33

1	<u>Blood Glucose (CHO) and Plasma pST levels (RIA) after</u>							
2	<u>Intra-Duodenal Infusion of Oral pST in Pigs</u>							
3								
4								
5		Pig-1		Pig-2		Pig-3		
6	Time(hr)	pST=0		pST=40ml		pST=20ml		
7		CHO*	RIA**	CHO	RIA	CHO	RIA	
8	-1	<2	<0.50	<2	1.86	2.2	1.51	
9	0	2.7	1.82	<2	0.71	<2	1.17	
10	1	2.5	1.75	2.2	2.48	2.2	0.98	
11	2	2.6	1.30	3.7	0.59	5.9	8.63	
12	4	2.2	0.65	3.2	3.18	3.4	2.72	
13	5	2.5	1.64	2.9	2.14	2.2	0.77	
14	6	2.4	1.30	3.3	2.30	3.2	1.29	
15	8	<2	<0.50	2.6	0.9	3.4	4.81	
16	10	3.9	-	3.1	2.61	4.8	1.07	
17	12	2.2	-	<2	0.85	2.9	1.06	
18	14	2.9	-	2.5	2.39	2.5	4.03	
19	24	-	-	-	0.71	-	4.29	

20

21

\*CHO=Blood Glucose Levels (mMol/l)

22

\*\*RIA=RIA measured plasma pST levels (ng/ml)

23

24

25

Blood Sugar and Plasma pST Levels after Intraduodenal  
Infusion of Oral pST

26

27

28

29

30

31

32

33

36

		Pig-4		Pig-5		Pig-6	
	Time(hr)	pST=10		pST=5ml		pST=5ml	
		CHO	RIA	CHO	RIA	CHO	RIA
1	-1	5.65	1.63	5.2	2.67	4	1.32
2	0	6.95	2.97	6.5	1.07	5.8	1.01
3	1	5.8	0.89	5.3	0.49	5.5	3.85
4	2	5.5	0.38	5.15	1.1	5.2	2.64
5	3	4.5	-	4.85	-	3.55	-
6	4	4.7	6.64	5.3	1.44	5.0	1.1
7	5	5.1	1.67	5.35	1.10	5.5	1.2
8	6	5.65	1.47	5.25	0.7	5.8	3.56
9	8	5.34	0.85	5.15	1.71	5.35	2.48
10	10	7.05	5.99	6.8	1.80	6.6	2.04
11	12	6.25	3.54	5.3	0.79	7.05	2.49
12	14	7.55	0.82	7.05	2.14	8.25	1.11
13	24	6.75	0.84	6.1	0.91	7.15	3.49
14	36	7.35	0.93	8.45	1.45	8.9	1.06

**BIOLOGICAL EXAMPLE D**

Using the formulation prepared in Example 4 (which involves a phospholipid-r-human growth hormone complex), the clinical bioactivity (measured as changes induced by oral r-hGH on blood sugar levels) and bioavailability of orally-administered r-hGH were studied in nine young, healthy male volunteers.

1 Table: Topography of Study Subjects (all male subjects)  
2

---

3	4	5	6	7	8
	Name	Age	Height (cm)	Weight (kg)	Oral r-hGH Dose (mg)
9	JBL	26	172	66	7
10	PJG	22	179	68	7
11	NMH	20	178	60	15
12	CSB	24	175	65	15
13	KKN	22	172	57	30
14	CSK	20	172	57	30
15	CYG	25	174	60	PLACEBO
16	KJH	27	175	65	PLACEBO
17	YKS	20	175	60	PLACEBO

---

18 Changes in blood sugar and hGH levels induced by oral administration of oral  
19 r-hGH in these 9 subjects at doses of 0, 7, 15, and 30 ml (0, 7, 15, and 30 mg  
20 of oral r-hGH formulation) were measured. Measurements were made by  
21 commercial kits on EDTA-treated plasma. Results for each subject are  
22 illustrated individually in Figure 1 to 9 and the average dose-responsiveness to  
23 the oral administration of r-hGH on RIA-measured plasma hGH is illustrated in  
24 Figure 10. A 'diabetogenicity' effect and an RIA-measurable elevation in  
25 plasma hGH were observed and, in all volunteers treated with active drug, such  
26 changes were 'biphasic' as has been observed with the administration of other  
27 formulations in accordance with this invention, such as oral pST in pigs and oral  
28 insulin in diabetics.

29  
30 Once again, apparently, the r-hGH administered orally (as phospholipo-r-hGH)  
31 is absorbed and induces the biological actions of hGH in humans. It is  
32 bioavailable in circulating blood usually at between 0.5 to 4 hours after oral  
33 dosing; the hGH is believed to be channelled into the liver, from where it is

released and available at the circulating blood once again after some 8 to 12 (or more) hours from oral dosing. This biphasic effect in hGH bioavailability (and additionally in bioactivity) is apparently dose-dependent: at relatively lower doses, oral hGH was bioavailable within 0.5 to 2 hours after oral dosing, and once again bioavailable after 8 hours or so; whereas at higher dose (30 mg of r-hGH), the initial peak bioavailability occurred at about 4 hours and the second peak was observed after 11 hours from the oral dose.

A recommended possible dose was 15 mg per man. In our study, 7 mg was also a significantly effective dose. 1 to 10 mg per man, in particular 3-5mg per man may be a suitable therapeutic dose.

#### BIOLOGICAL EXAMPLE E

The formulation of Example 5 (oral delivery form of salmon calcitonin: ODDS-sct) was studied in a group of young male volunteers. The demography of these volunteers is as follows:

Subject Code	Age (years)	Weight (kg)	Height (cm)	Blood Pressure(mmHg) Systolic/Diastolic	Pulse Rate beats/min
A (10)*	23	78	187	120/80	72
B ( 5)*	23	73	183	140/100	56
C (100)+20		59	172	110/70	56
D (10)*	25	61	173	100/60	60
E (100)+25		66	169	120/80	64
F ( 5)*	23	63	174	100/60	60

(\*Number of ODDS-sct capsules, each capsule containing 60 IU)  
(+IU of salmon calcitonin injected, subcutaneously)



1 Briefly, each subject was fasted over-night and either the ODDS-sCT capsules  
2 were administered, per os, or CALSYNAR<sup>TM</sup> (an injectable salmon calcitonin  
3 preparation) was administered subcutaneously at 6:00 a.m. of the study day.  
4 Venous blood samples were collected through an indwelling catheter in a  
5 forearm vein at time 0, 60, 90, 120, 150, 180, 210, 240, 300, and 360 minutes  
6 after the administration of testing medication. Serum phosphate levels were  
7 measured, immediately after collecting the blood samples, while plasma salmon  
8 calcitonin levels were measured by radioimmunoassay on EDTA-treated plasma  
9 samples.

10  
11 A marked reduction in serum phosphate levels was observed in all subjects who  
12 had received either 300 IU or 600 IU of ODDS-sCT capsules, and such changes  
13 in serum phosphate levels after oral administration of the salmon calcitonin  
14 formulation of Example 5 were similar to those of subjects after subcutaneous  
15 injection of salmon calcitonin. 300 IU and 600 IU of orally administered  
16 ODDS-sCT are thus shown to have a broadly similar effect on serum phosphate  
17 level 100 IU of s.c. injected sCT. Formulations of the present invention are  
18 therefore highly effective.

19  
20 EXAMPLE 7

21  
22 A phospholipo-salmon calcitonin complex was made by mixing  
23 1,2-dimyristoyl-sn-glycerol-3-phosphatidic acid monohydrate, and  
24 L- $\alpha$ -phosphatidylcholine bimyristoyl (sodium salt) in the presence of aprotinin  
25 in 0.9% of isotonic saline solution, and a chemical 'interaction' was induced at  
26 room temperature for 30 minutes. The phospholipids, which are known to  
27 participate in the L- $\alpha$ -phosphatidylglycerol pathway of synthesis of  
28 phosphatidylcholine at the epithelium of the small intestine, apparently  
29 non-covalently bind at the lipophilic sites of salmon calcitonin, in vitro.

30  
31 The above phospholipo-salmon calcitonin complex is suspended and mixed with  
32 a solution containing a surfactant having an HLB value of 14 or more  
33 (polyoxy-40-stearate) in the presence of a viscosity increaser (thickener) and

1 'stabiliser' for the suspended phospholipocalcitonin (eg, less than 5%,  
2 preferably less than 2% of hydroxypropylcellulose). The pH was adjusted to  
3 around pH 2.0 or so using concentrated solutions of citric acid and ascorbic acid  
4 (although any other acidic pH adjuster(s) could be used).  
5

6 The solution yielded above is suspended into three to four volumes of oleic acid  
7 (or any other C<sub>16</sub> or higher fatty acid) in the presence of a surfactant having an  
8 HLB value of 4 or less. The C<sub>16</sub> or higher fatty acid(s) may act as a "volume  
9 expander", as well as possibly an enhancer for transmembrane absorption of the  
10 phospholipo-salmon calcitonin, and/or as an "enteric coating" for the  
11 phospholipo-salmon calcitonin. However, this oleic acid/surfactant combination  
12 is not absolutely essential.  
13

14 The following shows the actual chemical composition of the phospholipo-salmon  
15 calcitonin preparation of this example:  
16  
17

18	19	Weight (mg or gm)		Remarks
		Preferred	Optimal	
21	Part A:			
22	Salmon calcitonin	200-1500 mg	480 mg	Rorer*
23	1,2-dimyristoyl-sn-	50-1000 mg	500 mg	
24	glycerol-3-phosphotidic			
25	acid monohydrate			
26	L-alpha-phosphocholine	50-1000 mg	500 mg	
27	bimyristoyl (sodium			
28	salt)			
29	Aprotinin	250-50000mg	10 gm (12 TIU.mg)	
30	Isotonic saline solution	50-400 mg	150 gm	

## 1    Part B:

2    Hydroxypropylcellulose	500-7200 mg	1.5 gm
3    Polyoxy-40-stearate	2-12 gm	4 gm
4    Citric acid	1-5 gm	2.4 gm
5    Ascorbic acid	1-7 gm	3.0 gm

6

## 7    Part C:

8    Oleic acid	125-1200 gm	540 gm
9    Tween-80	5-42 gm	10.5gm
10    Glycerolmonooleate	7.3-124.2gm	41.4gm

11

12            (\*Rorer salmon calcitonin, Lot # NPP 209; 8908047)

13

14    The procedure of making the formulation was as follows:

15

16    Part A was thoroughly mixed and left to stand at room temperature for 30  
17    minutes or more. Part B was mixed and prepared and then Part A was  
18    suspended in Part B under gentle stirring at room temperature. The pH was  
19    adjusted with citric acid and ascorbic acid to about pH 2.0.

20

21    Part C, the oil solution, was prepared by mixing. With gentle stirring, the Part  
22    A and Part B preparation was poured into Part C.

23

24    This mixed solution may be spray coated over approximately the same weight of  
25    powder consisting either of carboxymethyl-cellulose-Ca and alginic acid or of  
26    alginic acid and gelatin. The coated dried powder may be packed into a hard  
27    gelatin capsules and may be orally administered to human subjects/patients.  
28    Alternatively, the liquid form            may be orally administered either in a soft  
29    gelatin capsule or by itself.

30

31

32

33

BIOLOGICAL EXAMPLE F

Six young healthy male volunteers participated in this study. After an overnight fasting, at 05:30am the Example 7 salmon calcitonin preparation was orally administered to four subjects: two subjects received 300 IU of salmon calcitonin capsule as in Example 7, and another two subjects received 600 IU of oral salmon calcitonin as in Example 7. Two subjects were given CALCYNAR, a trade mark for an injectable salmon calcitonin, 100 IU, subcutaneously. Serum phosphate and plasma calcitonin levels, measured by means of radioimmunoassay method, were taken at 30 minutes before medication, at the time of medication, and at 30, 60, 90, 120, 150, 180, 210, 240 and 300 minutes after medication.

Table: Changes in serum phosphate (% of the control) and RIA-measured plasma sCT (P g/ml) in men after oral salmon calcitonin (Example 7 formulation) and after subcutaneous injection of CALCYNAR:

Time(Min)		RIA-sCT		(P g/ml)		:		Serum Phosphate(-%Change)					
Subj.*A	B	C	D	E	F	A	B	C	D	E	F		
0	N	N	105	N	N	N	4.54	4.01	4.31	4.50	4.88	5.11++	
30	N	N	150	N	220	34	7.1	5.7	12.5	14.2	9.4	2.5	
60	N	N	100	N	100	94	10.6	17.2	15.8	20.7	1.8	7.2	
90	48	N	44	N	48	62	17.8	21.2	17.2	25.8	6.6	11.0	
120	116	N	19	N	40	70	22.0	36.2	17.0	28.4	9.2	8.2	
150	138	N	42	N	16	63	17.4	16.0	13.0	2.7	+7.0	3.3	
180	50	N	N	N	48	120	30.8	31.9	29.2	36.4	18.4	15.1	
210	N	N	N	N	N	70	30.4	30.9	35.0	32.4	16.2	19.2	
240	N	N	N	N	N	60	24.9	29.2	31.3	20.7	17.0	13.5	
300	N	N	N	N	20	56	18.4	28.2	27.2	27.8	19.7	17.03	
360	N	N	N	N	24	48	11.0	12.0	23.9	18.2	26.6	8.6	

```
1  *Subj= Subject
2  ++5.11 (at Time 0 Minute)= Serum phosphate level at the
3  control, baseline value in
4  mg/ml serum.
```

5    **Note:**    All % changes in the serum phosphate levels are  
6                in (-) "minus" values, except where  
7                indicated. (The + sign means an increase  
8                in serum phosphate level).

9

**10 Code for Study Medication Dosings:**

11           **Subject A = 600 IU oral sCT as the Example I**  
12           **formulation, per os;**

13            **Subject B = 300 IU, per os;**

14 Subject C = 100 IU CALCYNAR; subcutaneous  
15 injection;

16                      Subject D = 600 IU, per os;

17           **Subject E = 100 IU, CALCYNAR, subcutaneous**  
18                           **injection;**

19                      **Subject F = 300 IU, per os.**

20

21 The Example 7 formulation of salmon calcitonin given orally to a group of  
22 healthy male subjects was biologically active in reducing serum phosphate to an  
23 extent which was equal to or greater than that effect of subcutaneously injected  
24 salmon calcitonin in men. However, RIA-measurable plasma sCT was not  
25 always detectable by the present methodology applied after oral ingestion of  
26 either 600 or 300 IU of Example 7 salmon calcitonin. By binding sCT with  
27 phospholipids, certain antigenic changes in the sCT may be caused; the sCT  
28 may therefore not be detected by currently the sCT-specific antibodies used in the  
29 radioimmunoassay.

30

31

32

33

1    EXAMPLE 8

2

3    An orally administrable insulin (bovine insulin) preparation was made by  
4    mixing insulin with L- $\alpha$ -phosphocholine (lecithin precursor) in the presence of  
5    an appropriate surfactant and aprotinin in isobutyl alcohol (0.9%). The  
6    non-covalently bound complex thus formed was suspended into water and MCT  
7    (medium chain-length triglycerides) oil for subsequent oral administration to a  
8    group of diabetics.

9

10   The following is the chemical composition of the formulation:

11

12   Chemical IngredientsWeight (mg or gm)PreferredOptimal

13

14   Part A:

15   Bovine insulin

0.5 - 5 gm

1 gm

16   L-alpha-phosphocholine

50 - 2000 mg

500 mg

17   Aprotinin as TRASYLOL™

50 - 250 ml

150 ml

18   (5 ml = 100,000 KIU)

19   Citric acid

1 - 5 gm

2.4 gm

20   Ascorbic acid

1 - 7 gm

3.0 gm

21

22   Part B:

23   Polyoxy-40-stearate

2 - 12 gm

4.0 gm

24   Hydroxypropylcellulose

0.5 - 8 gm

1.5 gm

25

26   The formulation was prepared as follows:

27

28   Part A was mixed at room temperature thoroughly with gentle stirring. Part B  
29   was prepared by dissolving HPC and polyoxy-40-stearate completely in 100 ml  
30   of deionized water. Part A was then added to Part B, with gentle stirring and  
31   thorough mixing at room temperature.

32

33

This insulin formulation may be given orally to diabetics as it is, or it may be suspended into 10 to 30 ml of MCT (medium chain-length triglyceride) oil, which may act as an enteric coating and a volume expander, so as to promote gastric emptying of the formulation through the pylorus and into duodeno-jejuno-ileum.

#### BIOLOGICAL EXAMPLE G

Eight diabetic patients participated in this study. While fasting, early in the morning of the study, each subject took orally the Example 8 oral insulin formulation; the blood sugar was measured for two hours.

Name	Sex	Age	Wt(Kg)	Type	Dose (IU)	Blood Sugar (mMol/dl)		
						0	60	120(Min)
COJ	F	77	60	II	30	10.4	-	9.4
KDS	M	63	63	II	24	9.3	8.5	8.1
SDG	M	51	52	II	24	10.4	8.4	7.0
HKH	M	44	70	I	24	9.5	7.9	7.8
CYH	M	39	50	I	24	9.1	7.8	-
NCS	F	53	65	II	30	14.5	10.8	10.1
SKW	M	66	61	II	30	10.5	9.7	8.7
YYC	F	49	57	II	48	20.2	17.3	13.7

The insulin formulation of Example 8, when orally administered, was effective in controlling hyperglycemia of both diabetic types.

1 CLAIMS

2

3 1. A pharmaceutical formulation comprising a biologically active material  
 4 in association with lecithin or a compound capable of acting as a precursor for  
 5 lecithin *in vivo*.

6

7 2. A formulation as claimed in claim 1, wherein the biologically active  
 8 material is proteinaceous.

9

10 3. A formulation as claimed in claim 2, wherein the biologically active  
 11 formulation is insulin, erythropoietin, porcine somatotropin, human growth  
 12 hormone or salmon calcitonin.

13

14 4. A formulation as claimed in claim 1, 2 or 3, wherein the biologically  
 15 active material is present together with a lecithin precursor.

16

17 5. A formulation as claimed in claim 4, wherein the lecithin precursor is a  
 18 phospholipid.

19

20 6. A formulation as claimed in claim 5, wherein the phospholipid has the  
 21 general formula:

22

23

24

25

26

27

28

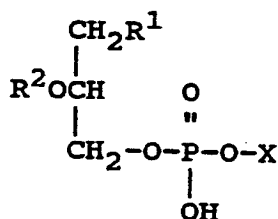
29

30

31

32

33



wherein each of R<sup>1</sup> and R<sup>2</sup> independently represents an acyl group of for  
 example 14 to 26 carbon atoms which is optionally mono- or poly-unsaturated  
 and X represents a hydrogen atom or a phospholipid head group.



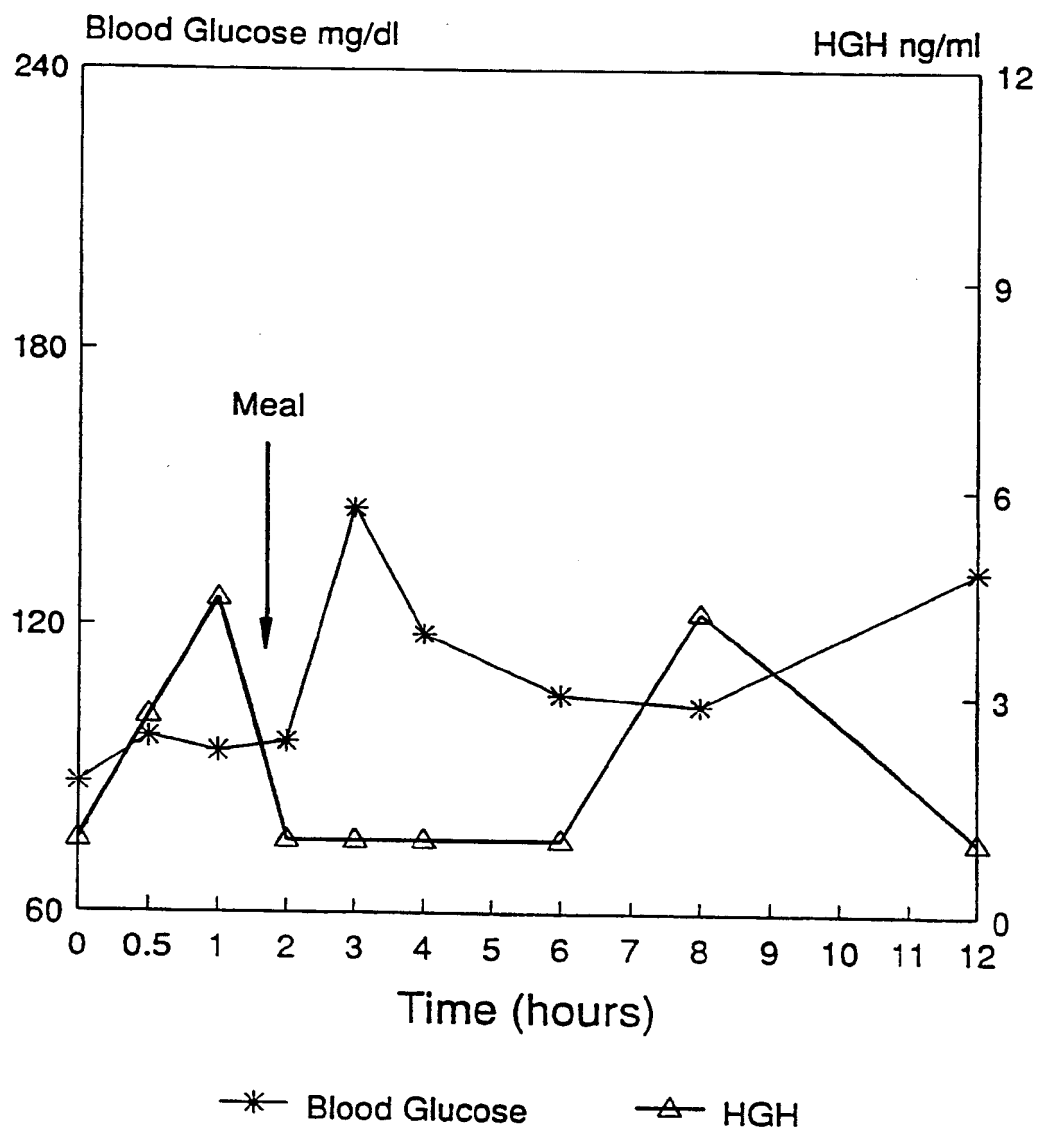
- 1 7. A formulation as claimed in claim 6, wherein the phospholipid head  
2 group is a residue of ethanolamine, choline, serine or glycerol.  
3
- 4 8. A formulation as claimed in claim 5, wherein the phospholipid  
5 comprises:  
6  
7 dimyristoyl phosphatidyl glycerol (DPMG),  
8 dipalmitoyl phosphatidyl glycerol (DPPG),  
9 distearoyl phosphatidyl glycerol (DSPG),  
10 dimyristoyl phosphatidylcholine (DPMC),  
11 dipalmitoyl phosphatidylcholine (DPPC),  
12 distearoyl phosphatidylcholine (DSPC),  
13 dimyristoyl phosphatidic acid (DPMA),  
14 dipalmitoyl phosphatidic acid (DPPA) or  
15 distearoyl phosphatidic acid (DSPA).  
16
- 17 9. A formulation as claimed in claim 5, wherein the phospholipid  
18 comprises:  
19  
20 dimyristoyl phosphatidyl ethanolamine (DPME),  
21 dipalmitoyl phosphatidyl ethanolamine (DPPE), and  
22 distearoyl phosphatidyl ethanolamine (DSPE).  
23
- 24 10. A formulation as claimed in claim 1, which comprises lecithin.  
25
- 26 11. A formulation as claimed in any one of claims 1 to 10 comprising a  
27 hydrophilic liquid.  
28
- 29 12. A formulation as claimed in any one of claims 1 to 11, comprising a  
30 hydrophilic surfactant.  
31
- 32 13. A formulation as claimed in any one of claims 1 to 12, comprising a  
33 protease inhibitor.

- 1 14. A pharmaceutical formulation as claimed in any one of claims 1 to 12  
2 comprising a lipophilic phase and a hydrophilic phase.  
3
- 4 15. A formulation as claimed in claim 14, wherein the lipophilic phase  
5 comprises:  
6  
7 one or more oils (such as cholesterol or any other material that forms a  
8 chylomicron matrix);  
9  
10 lecithin or any other useful phospholipid; and  
11  
12 a lipophilic surfactant.  
13
- 14 16. A formulation as claimed in any one of claims 1 to 15, comprising a  
15 stabiliser for the biologically active material.  
16
- 17 17. A formulation as claimed in claim 14 or 15, comprising an  
18 emulsification aid.  
19
- 20 18. A formulation as claimed in any one of claims 1 to 17, comprising a  
21 stabiliser and/or plasticiser.  
22
- 23 19. A formulation as claimed in any one of claims 1 to 18, comprising a  
24 preservative.  
25
- 26 20. A formulation as claimed in claim 19, wherein the preservative  
27 comprises an antioxidant.  
28
- 29 21. A formulation as claimed in claim 19 or 20, wherein the preservative  
30 comprises an antimicrobial.  
31
- 32 22. A formulation as claimed in any one of claims 1 to 21, which is a solid.  
33

- 1 23. A process for the preparation of a pharmaceutical formulation as claimed  
2 in any one of claims 1 to 22, the process comprising admixing the ingredients.  
3
- 4 24. A process for preparing a pharmaceutical composition the process  
5 comprising:  
6
- 7 (i) providing a lipophilic phase;  
8
- 9 (ii) providing a hydrophilic phase comprising a biologically active  
10 substance and lecithin or a lecithin precursor; and  
11
- 12 (iii) forming a two-phase system by intimate admixture of the lipophilic  
13 and hydrophilic phases.  
14
- 15 25. A process as claimed in claim 23 or 24, wherein the formulation is  
16 converted into a solid by freeze drying.  
17
- 18 26. A process as claimed in claim 23 or 24, wherein the formulation is  
19 converted into a solid by spraying onto carrier granules or particles.  
20
- 21 27. A method of treating a human or other animal, comprising the oral or  
22 rectal administration of a formulation as claimed in any one of claims 1 to 22  
23 and/or produceable by a process as claimed in any one of claims 23 to 26.  
24
- 25 28. The use of the ingredients of a formulation as claimed in any one of  
26 claims 1 to 22 and/or produceable by a process as claimed in any one of claims  
27 23 to 26 in the preparation of an orally or rectally administrable formulation for  
28 the treatment or prophylaxis of disorders treatable or controllable by a  
29 biologically active material.  
30  
31  
32  
33

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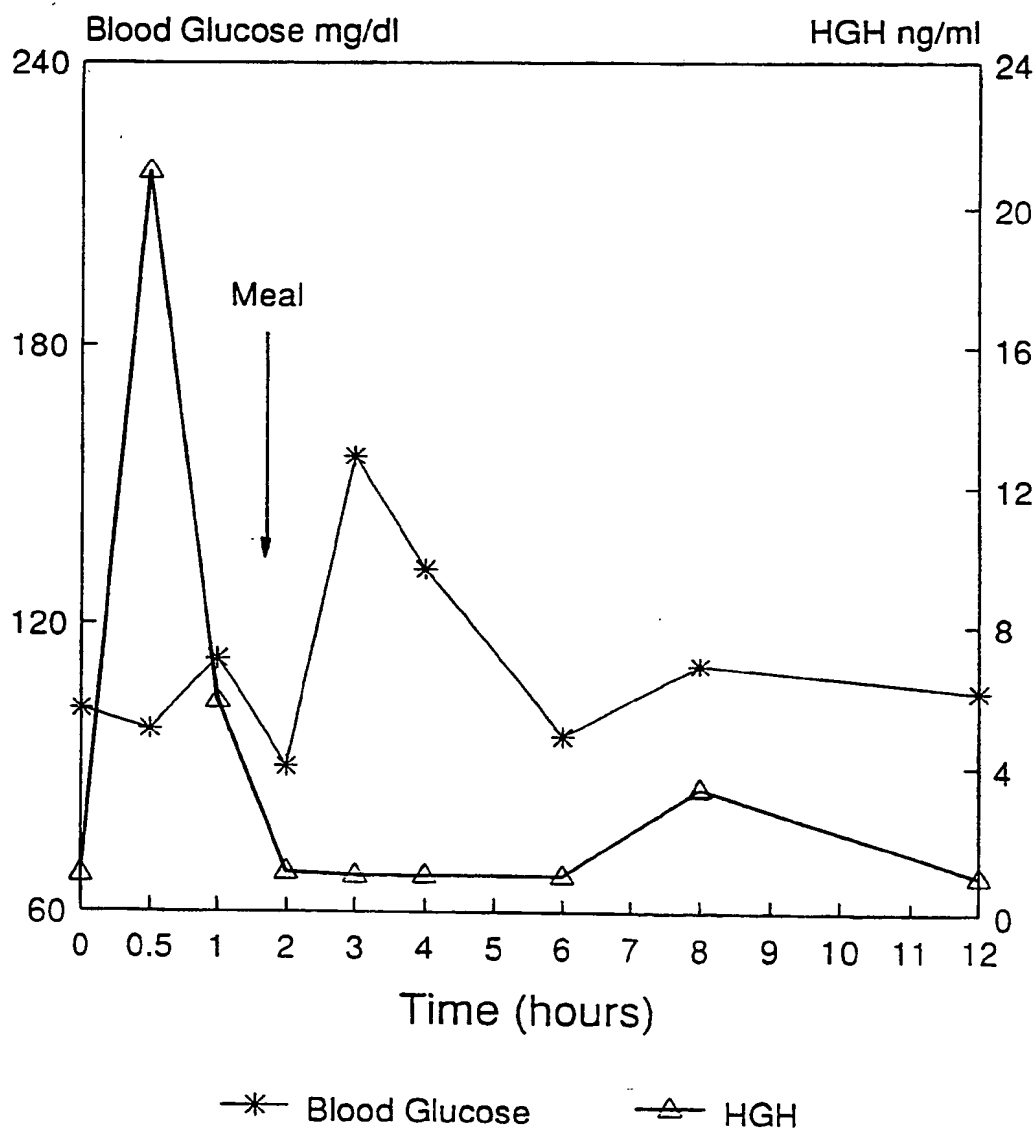
FIG. 1  
HGH and Blood Glucose Levels  
Subject JBL : 7 ml



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**FIG. 2**  
HGH and Blood Glucose Levels  
Subject PJG : 7 ml

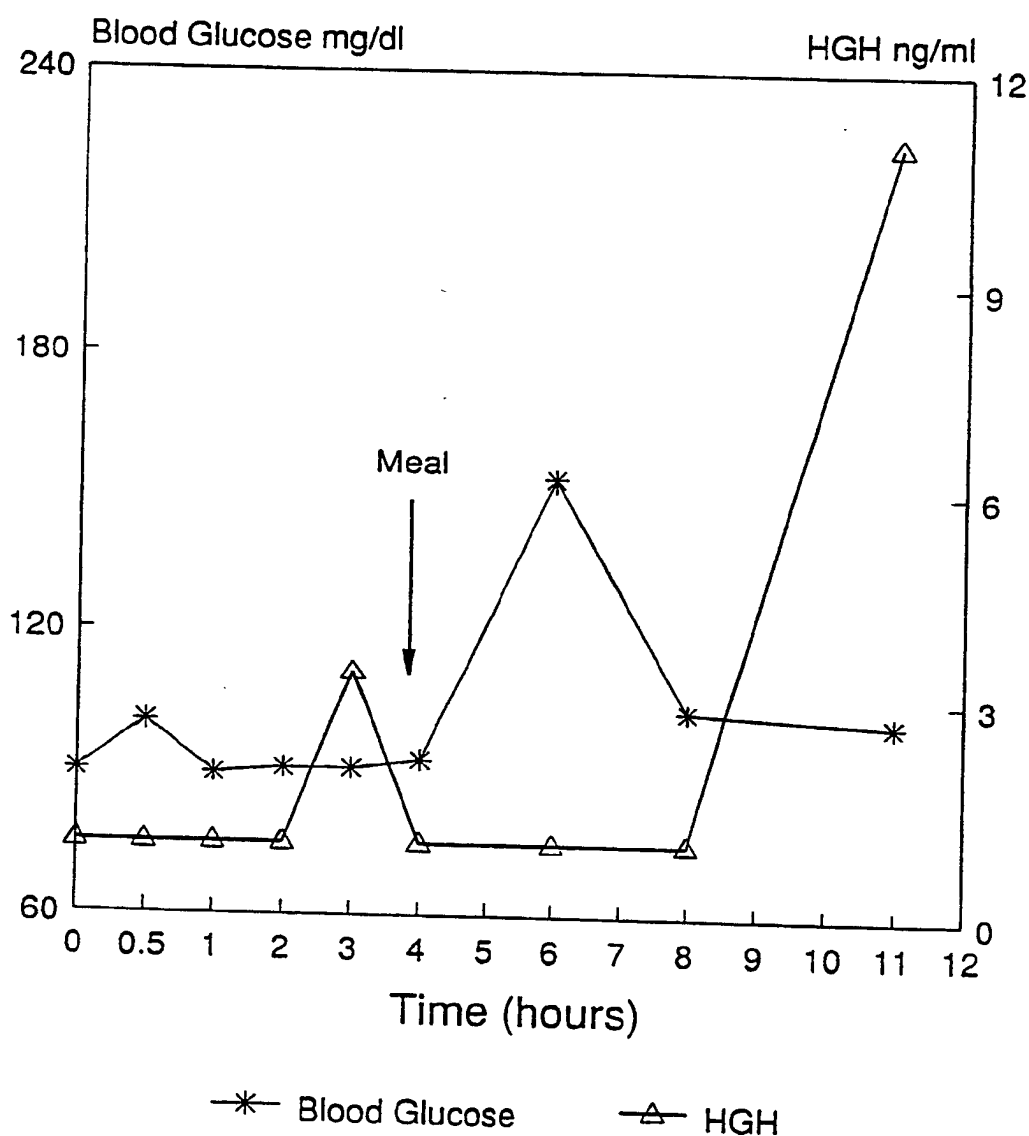


(Note HGH Scale Change)

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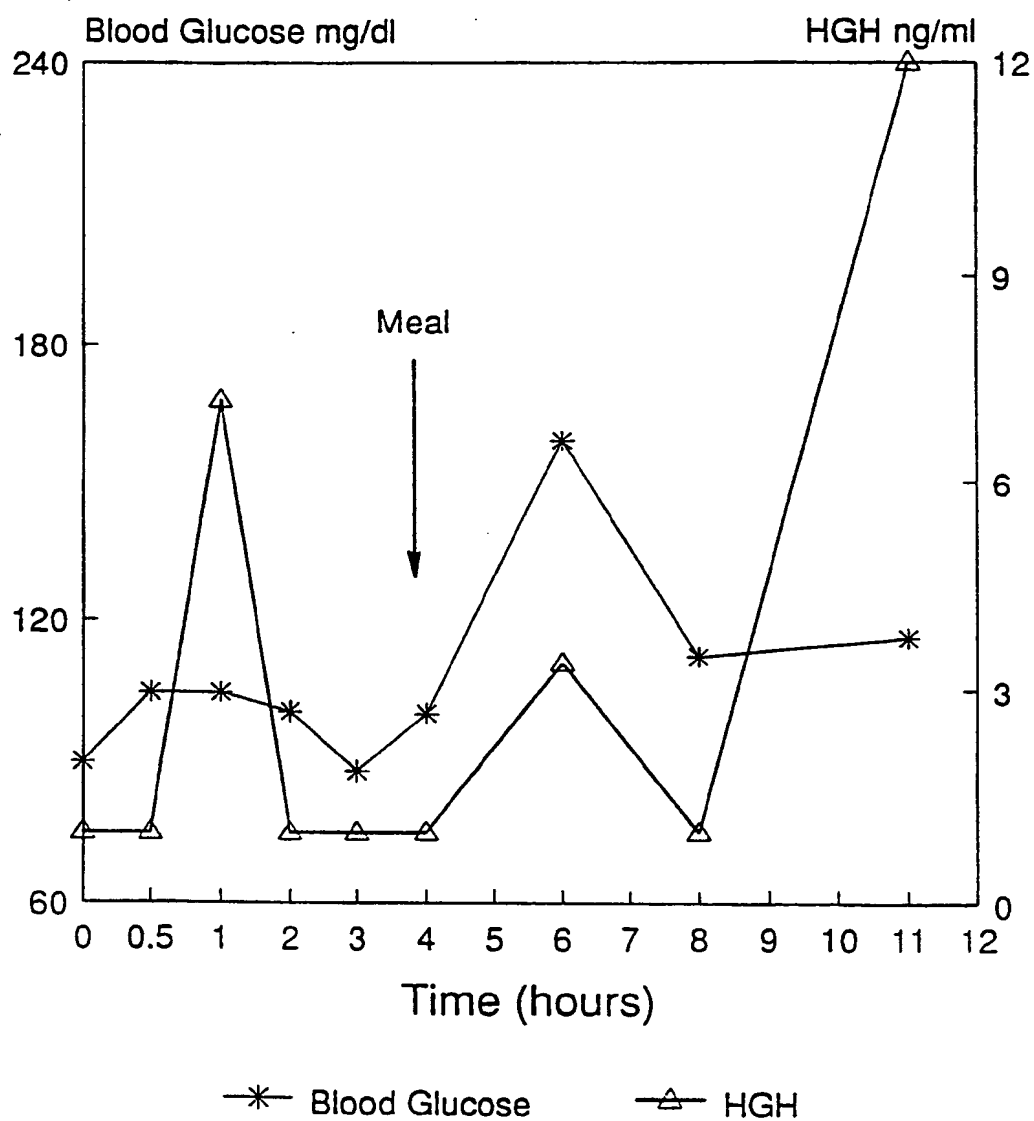
**FIG. 3**  
HGH and Blood Glucose Levels  
Subject NMH : 15 ml

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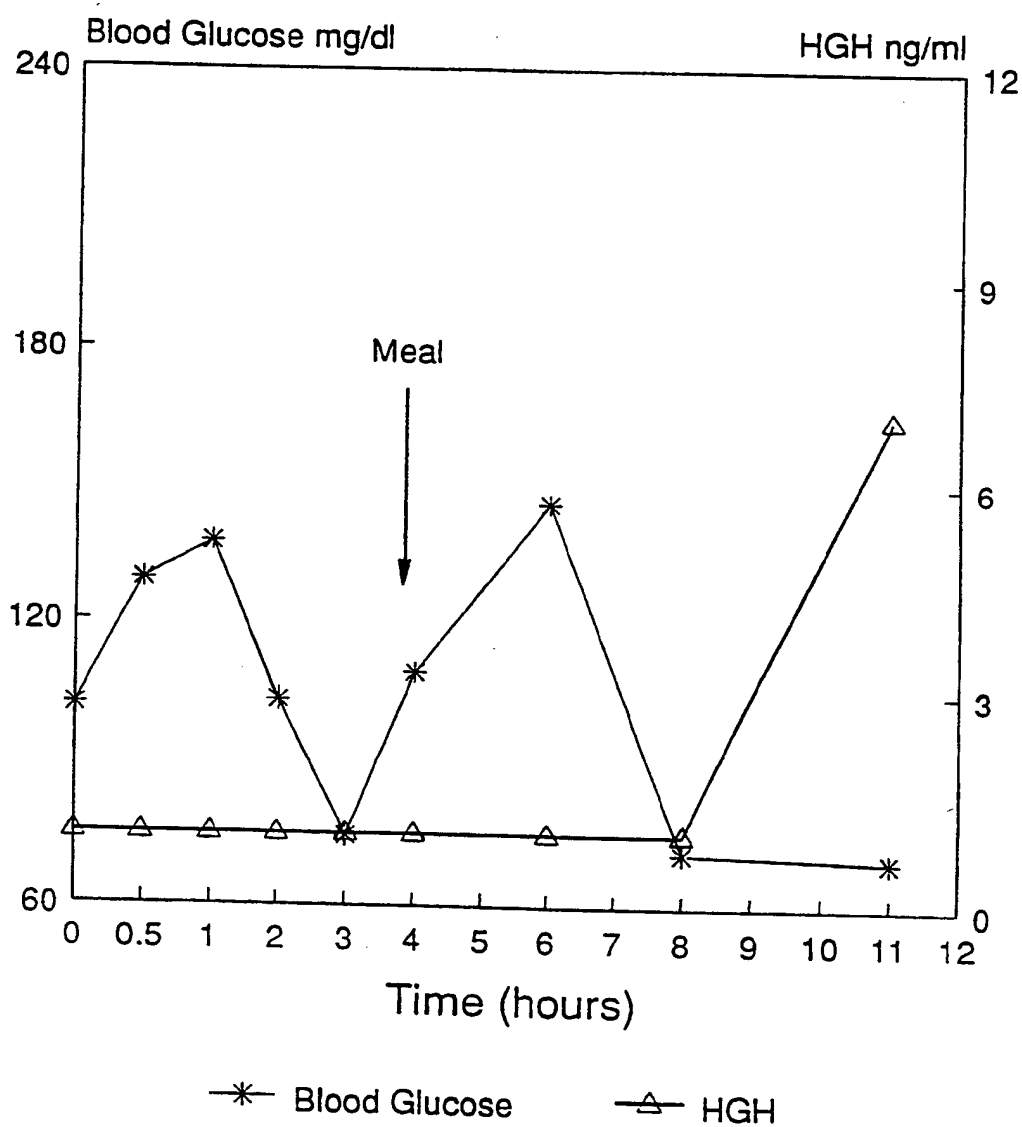
## FIG. 4

HGH and Blood Glucose Levels  
Subject CSB : 15 ml



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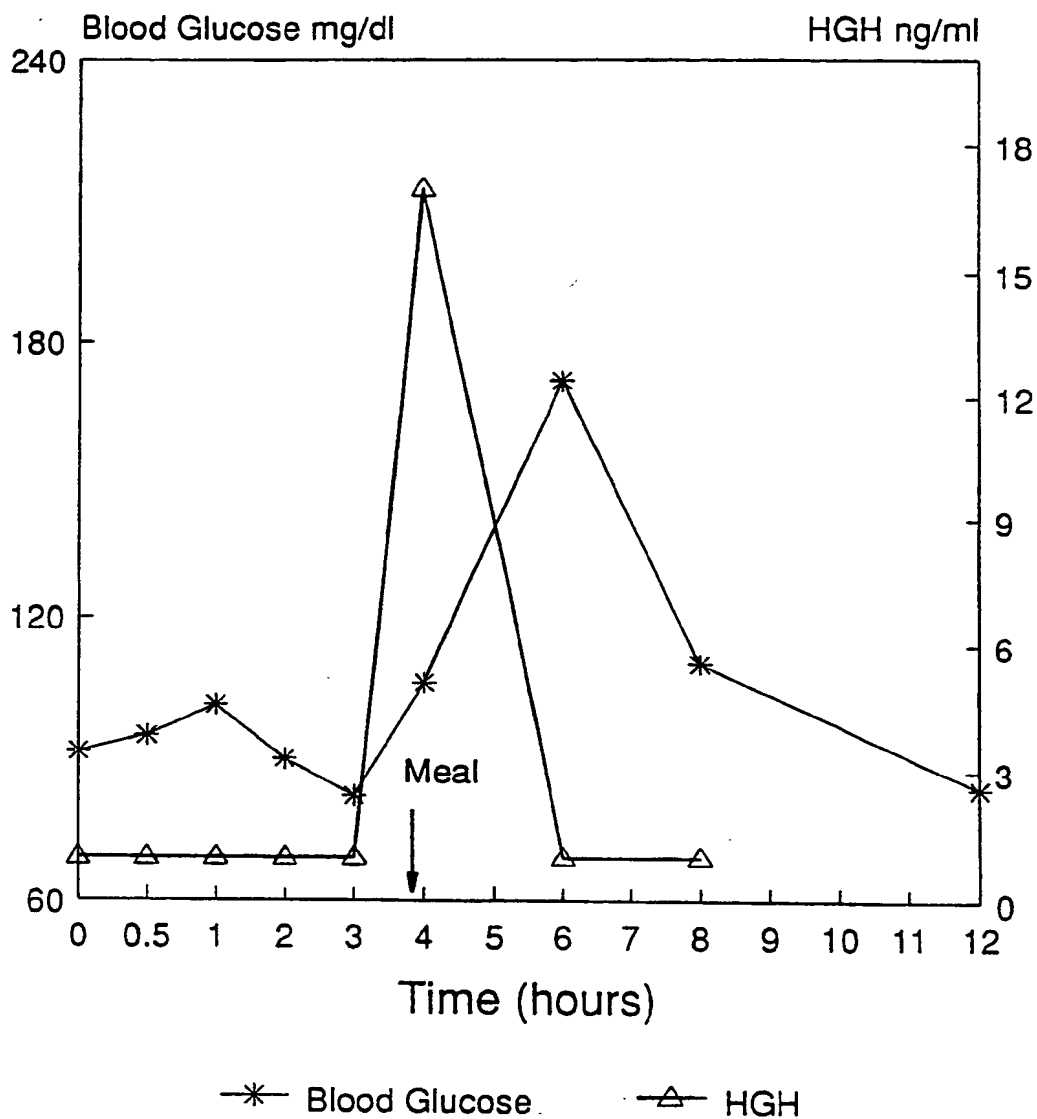
**FIG. 5**  
HGH and Blood Glucose Levels  
Subject KKN : 30 ml





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**FIG. 6**  
HGH and Blood Glucose Levels  
Subject CSK : 30 ml

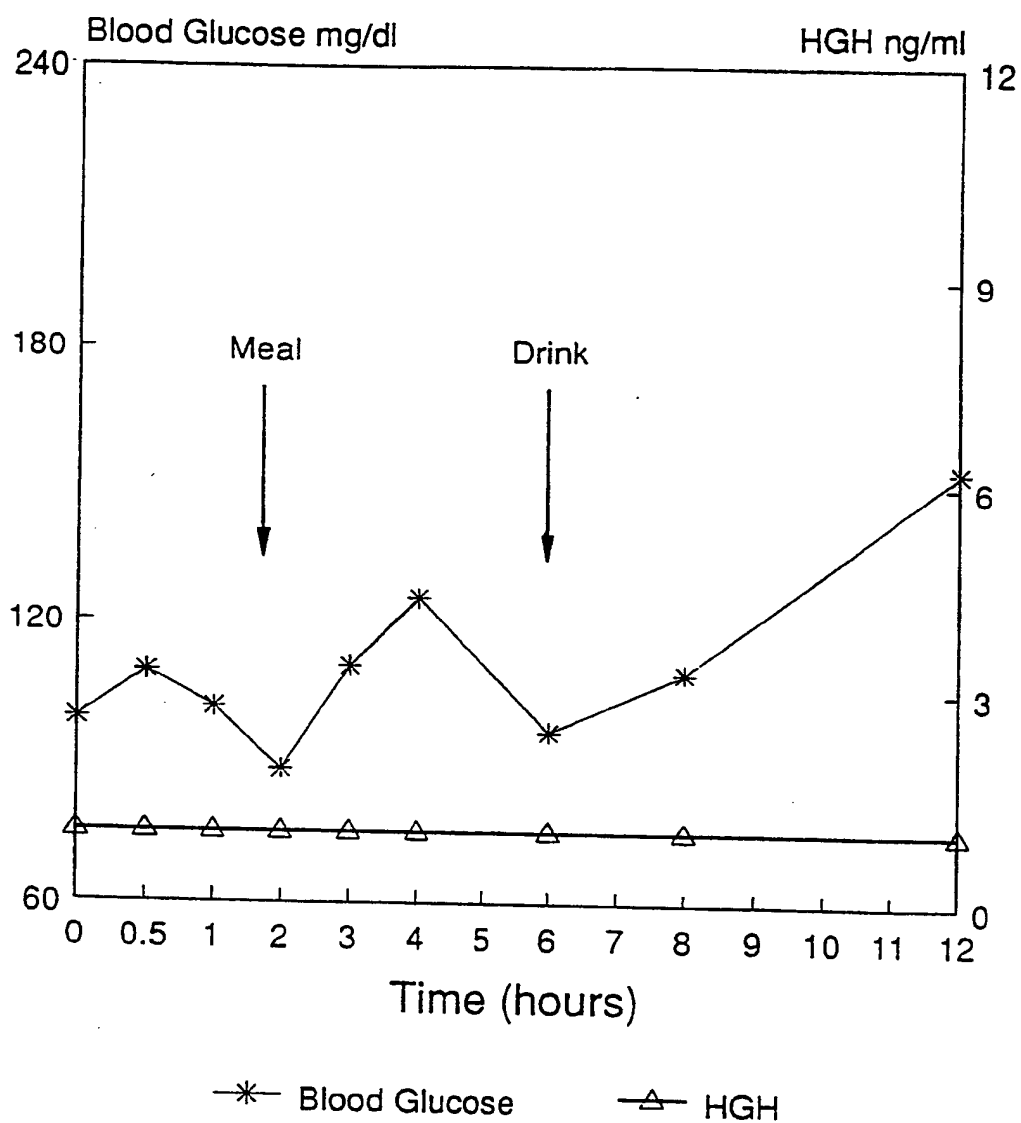


(Note HGH Scale Change)

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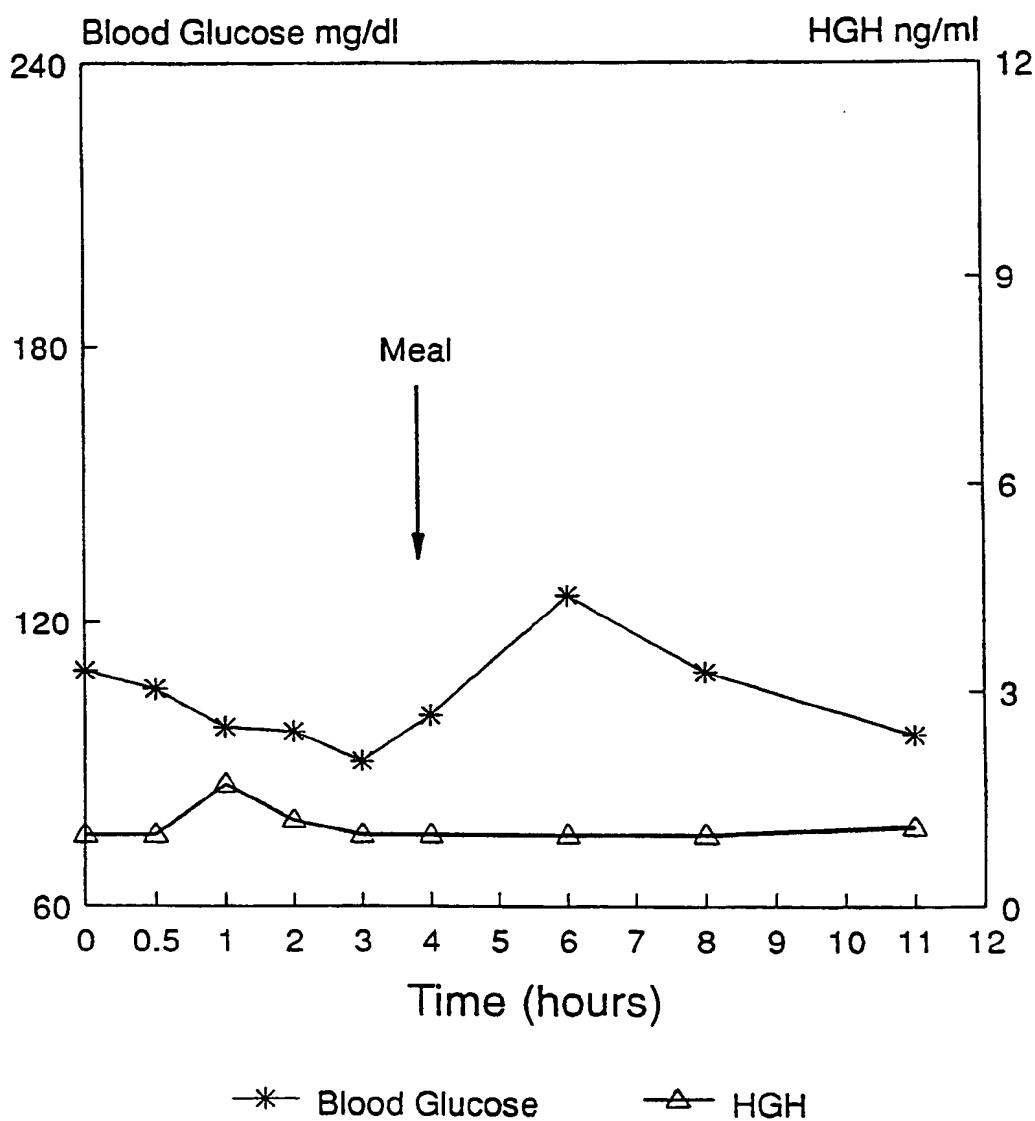
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**FIG. 7**  
HGH and Blood Glucose Levels  
Subject CYG : Control

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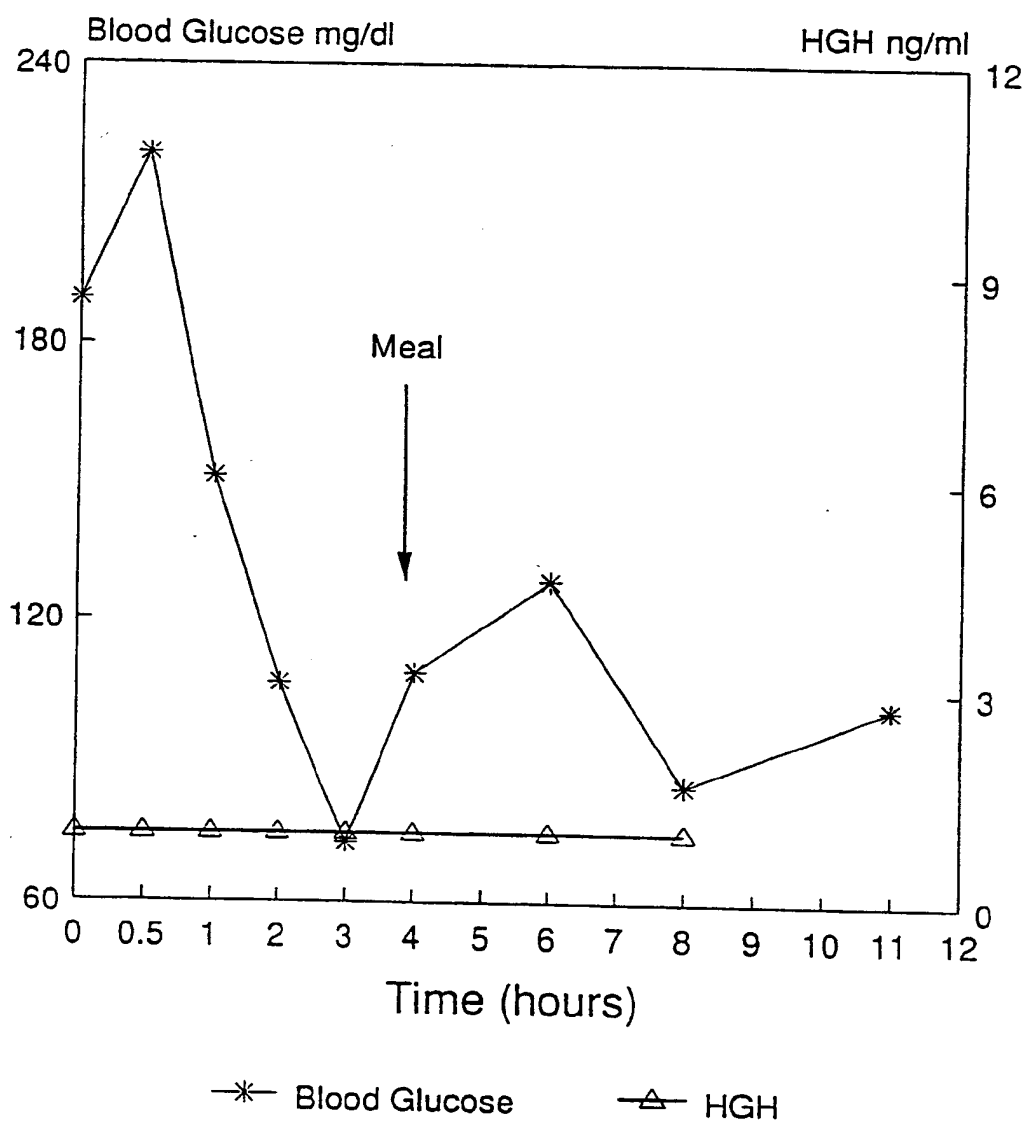
## FIG. 8

HGH and Blood Glucose Levels  
Subject KJH : Control

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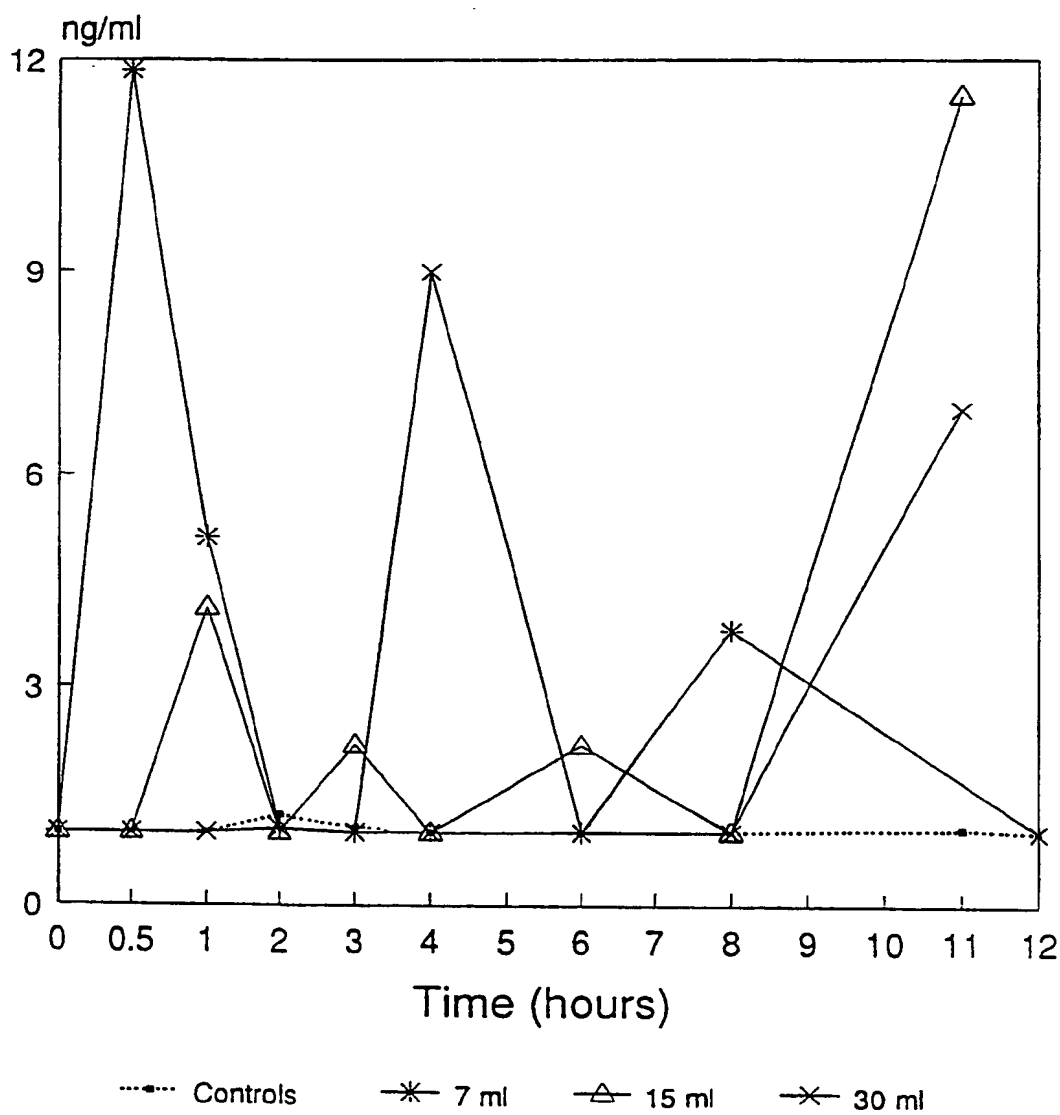
9/10

**FIG. 9**  
HGH and Blood Glucose Levels  
Subject YKS : Control

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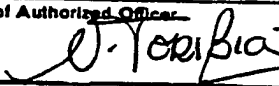
## FIG. 10

HGH Levels after administration of an  
Oral Formulation of HGH

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# INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 91/00510

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) * According to International Patent Classification (IPC) or to both National Classification and IPC IPC <sup>5</sup> : A 61 K 47/24, 9/107		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
IPC <sup>5</sup>	A 61 K	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched *		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT *</b>		
Category *	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
X	GB, A, 1180996 (INST. DE RECHERCHES CHIMIQUES ET BIOLOGIQUES APPLIQUEES) 11 February 1970 see the whole document --	1,2,4-7,9
X	EP, A, 0317120 (VESTAR, INC.) 24 May 1989 see page 4, line 19 - page 6, line 47 --	1,5-8,11, 22,23
X	GB, A, 2107985 (NOVO INDUSTRI) 11 May 1983 see the whole document --	1-11,18-21, 23
X	FR, A, 2581543 (TRESSENS) 14 November 1986 see the whole document --	1-7,9,11, 22,23,25,28
X	EP, A, 0140085 (FUJISAWA PHARM. CO.) 8 May 1985 see page 1, line 1 - page 6, line 34; page 9, example 3; page 10, example 5 cited in the application -- ./.	1-3,10-12, 14,15,22-26
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents: <sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p> </div> </div>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search		Date of Mailing of this International Search Report
1st July 1991		02.08.91
International Searching Authority		Signature of Authorized Officer
EUROPEAN PATENT OFFICE		 MARIA TORIBIO

## FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X	GB, A, 2085729 (DAINIPPON CO. LTD) 6 May 1982 see the whole document --	13,28
X	US, A, 4164573 (GALINSKY) 14 August 1979 see the whole document; in particular column 3, lines 28,29 --	28
A	WO, A, 8705505 (EURASIAM LAB. INC.) 24 September 1987 see page 44, example 6; claims -----	1-26,28

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE <sup>1</sup>

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers 27, because they relate to subject matter not required to be searched by this Authority, namely:

see PCT - Rule 39.1(iv): methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. ☐ Claim numbers \_\_\_\_\_, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers \_\_\_\_\_, because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING <sup>2</sup>

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

## Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

GB 9100510

SA 46139

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 24/07/91. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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GB-A- 2085729	06-05-82	JP-A- 57070814 DE-A- 3141223 FR-A, B 2492260 US-A- 4348384	01-05-82 24-06-82 23-04-82 07-09-82
US-A- 4164573	14-08-79	None	
WO-A- 8705505	24-09-87	AU-B- 608756 AU-A- 7207387 EP-A- 0302065 US-A- 4849227	18-04-91 09-10-87 08-02-89 18-07-89



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